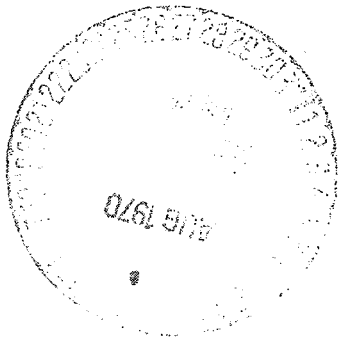
FINAL REPORT

2 VOLUMES



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CALIFORNIA INSTITUTE OF TECHNOLOGY
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FEASIBILITY STUDY FOR COMBINED METHOD OF STERILIZATION

FINAL REPORT

2 VOLUMES

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SUMMARY

The feasibility of multimethod sterilization was evaluated with respect to reduction of treatment severity while maintaining sterilization reliability. Appropriate literature was collected and abstracted. An interlocking key to 187 abstracts was prepared for ready reference to 16 topics relating to sterilization. Parameters modifying the biological action of ionizing radiations, ultraviolet radiation, thermal resistance, chemicals, electric fields, magnetic fields and ultrasonics were discussed. Reports which concern simultaneous or consecutive application of these agents were of particular interest. Advantages and limitations of these treatments were noted with regard to their application in spacecraft sterilization.

INTRODUCTION

Spacecraft which are to land on or impact Mars are to be sterilized prior to launch because of various biological considerations associated with detection of life on Mars. Heat is specified at present for internal sterilization. The requirement that the spacecraft be maintained for 24 hours at 135 C, and the time necessary for warm-up and cooling present problems involving scheduling, repairs, and reliability of components (6, 13, 15, 16, 169). Other methods of sterilization also have limitations when considered individually. On the other hand, a combination of two or more treatments, each of lesser severity than would be required if applied alone, may sterilize in an additive manner. Furthermore, two agents may interact so that a synergistic effect can be demonstrated by a degree of microbial mortality well in excess of that which could be attributed to each agent. A combination of treatments which displays either of these relationships would eliminate or minimize the disadvantages of the individual treatment required to effect sterility.

The object of this contract is to consider the feasibility of multimethod sterilization so that the severity of treatment may be decreased while maintaining sterilization reliability. Although

dry heat has been favored as a sterilizing agent, there were no restrictions on possible agents and combinations within the constraints of material tolerance and material penetration.

The work centered around collection and evaluation of the literature. Selected articles have been abstracted and given an entry number. Although most articles have been abstracted in accordance with the abstracting system described in Progress Report No. 2, April 1-30, 1964, certain papers have been abstracted to include extensive experimental or conceptual data.

This report includes 187 abstracts and a key to the abstracts which permits immediate determination of those abstracts which concern several of 16 topics relating to sterilization, subject and author indexes, a discussion of the literature, and recommendations for future work. Some citations which consider the key position of spacecraft sterilization in formulation of national and international policy for extraterrestrial exploration have been included (1, 2, 5-8, 10, 11, 13, 14, 16-22).

The library services of Mary Ann Archer and Linda L. Brooks, and the secretarial work of Virginia Kay Allen are acknowledged.

Norman S. Davis

IONIZING RADIATIONS AND PARAMETERS MODIFYING RADIATION STERILIZATION

The ionizing radiations include alpha, beta, gamma and X-rays, high energy electrons, protons, and neutrons. These radiations are termed ionizing because the primary means of energy dissipation is the ejection of electrons from atoms of the material through which it passes. Gamma rays and X-rays are deeply penetrating, and have similar effects on cells. They are the principal ionizing radiations studied in microbial systems. Although ionizing radiation is ordinarily considered destructive, the background radiation in our environment may be necessary for normal life processes (90).

Aerobic and anaerobic bacterial spores resist higher doses of radiation than vegetative cells. Radiation resistance is not uniform within a cell population (144) or among strains of the same species (96, 101, 108). Differences in surface properties make it possible to separate radiation resistant and nonresistant cells in a culture (94). Inactivation is essentially logarithmic with increasing radiation doses (96), but occasional spores may survive extreme radiation doses (9 Mrad) although the progeny of such survivors do not have unusual radiation resistance (97). A computer program has been designed for mathematically studying

radiation effects on cell populations (89).

The chemical and physical environment prior to, during, and after irradiation can influence the radioresistance of an organism (95, 109, 110, 116, 127, 176). Thus the environment can afford protection (72, 106, 123, 130, 131, 137, 138, 139) or increase the sensitivity of cells to ionizing radiation (72, 92, 106, 136, 142).

The nutritional environment after irradiation (119, 131) is also critical for expression of radioresistance since the requirements for resistant cells to initiate growth may be different from the requirements of unirradiated cells. A cell may be injured by radiation, fail to grow in ordinary culture media, yet maintain viability. Stapleton (119) and Stapleton, Engel and Orce (50) showed that cultural conditions influence the resistance of Escherichia coli to physical and chemical agents. Resistant cells of strain B/r (CSH) require certain amino acids in the post-irradiation plating media for expression of resistance. Since any sterilization process depends on absence of growth in culture media as an indication that a reliable process has been applied, a large number of injured but non-multiplying organisms would still be significant unless it can be shown that the likelihood of a favorable extraterrestrial environment is remote.

Radiation Inactivation. The threshold sterilizing dose for 10^4 Clostridium botulinum spores in neutral phosphate buffer was determined by Anellis and Koch (108) to be 1.4 Mrad. About 15 of 102 strains had partial survival at this dose of gamma rays. The most resistant strains had D values of 0.317 to 0.336 Mrad. The sterilizing efficiency of gamma radiation is additive with increments of radiation (113).

Nickerson, Proctor and Goldblith (87) found 1.5-2.0 X 10^6 rep cathode rays were required to sterilize foods inoculated with 160-1800 Clostridium sporogenes spores per g.

Duggan, Anderson, and Elliker (106) reduced the viability of some radiation resistant vegetative bacteria by a factor of about 10^{-5} with 3.0 Mrad and by a factor of over 10^{-9} with 4.0 Mrad.

Grecz, Walker and Anellis (114) found that the substrate composition influenced the shape of the gamma radiation survival curve for Clostridium botulinum after 0.7 and 0.9 Mrad administered over the temperature range -196 to +90 C. Depending on the substrate and dose, radiation resistance appeared lowest at -20 to +40 C. Grecz and his associates (115) observed that radiation resistance of C. botulinum was greater at -196 C than at 0 C. Doses of 3.9 and 3.0 Mrad were required to inactivate 5 X 10^4 spores at -196 and 0 C respectively.

Roberts (117) reported that young encysting cells of Azotobacter vinelandii are sensitive to low radiation doses. Gamma and X-ray doses of 30-100 rad inactivated 10-30 percent of the cells. About 90 percent were inactivated by 6,000 rad. This organism and Escherichia coli K12 (lambda) (118) are potential biological indicators of low radiation doses.

Drake, Evans, and Niven (65) determined the radiation resistance of spores of several cultures isolated from irradiated canned ham. The LD_{99} values were: Bacillus licheniformis, 249,000 rad; Bacillus coagulans, 338,000 rad; Bacillus cereus, 513,000 rad. The radiation resistance of Streptococcus faecium strain HS-5 was as high as most bacterial spores.

Exposure of Clostridium roseum spores to 350 kiloroentgens was found by Wooley, Collier, and Clark (146) to prevent outgrowth of 90 percent of the irradiated spores.

Etchells et al. (97) found that 3 Mrep were required to sterilize small cucumbers. One to ten spores usually survived 1-2 Mrep. Other bacteria, molds and yeast had few survivors after exposure to 500,000 - 750,000 rep. Radiation resistance of cultures has been increased (101) by successive irradiations, but Webb and Clark (151) obtained a significant increase in sensitivity of Nocardia corallina coccoids by successive X-ray irradiation.

Temperature. Efforts to lower the dose required for radiation sterilization of foods have resulted in numerous papers concerned primarily with chemical sensitizers and temperature control. The chemical applications would be of questionable value in spacecraft sterilization since the necessary aqueous environment would not subject internal portions of spacecraft components to the chemical.

Graikoski (75) studied the interaction of temperature and gamma irradiation on spores of Clostridium botulinum and putrefactive anaerobe NCA 3679. These spores were slightly more resistant to radiation at -70 than at 4 C. Spore survival was greatest at irradiation temperatures just below the termal lethal threshold. Above this threshold, 85 C for Clostridium botulinum, spores were rapidly inactivated by radiation. Survivors increased in number as the irradiation temperature was raised above room temperature. Although radiation sensitized spores to heat, the thermal lethal threshold was not lowered.

Graikoski noted that irradiation should be conducted at the thermal lethal threshold of the most heat resistant organism in order to evaluate this combined method of sterilization. He concluded that the best procedure for sensitizing spores to heat would utilize heating prior to irradiation, during irradiation, and after irradiation at a lethal temperature. Heat and irradiation must be applied simultaneously to demonstrate the temperature effect.

Bellamy, Erickson and Lynch (98) irradiated spores over the range -178 to 110 C with 1.5 MeV electrons. The resistance of Bacillus pumilis spores irradiated anaerobically was nearly independent of temperature between -178 and +72 C, but decreased linearly at irradiation temperatures from 72 to 100 C. Bacillus megaterium was least resistant at 0 C. Aerobically irradiated Bacillus megaterium spores do not, however, display a minimum sensitivity over the irradiation temperature range -70 to +80 C. Dried spores are much more radiation resistant than spore suspensions at elevated temperatures.

Nickerson, Proctor and Goldblith (87) obtained greater radiation resistance in C. sporogenes irradiated in nitrogen, vacuum, frozen and in vacuum, and frozen, than at room temperature in air. Licciardello and Nickerson (72) required less radiation to kill 90 percent of Clostridium sporogenes PA 3679 spores at 20 C than at 66-68 C or -78 C.

Duggan, Anderson and Elliker (106) did not observe differences in resistance when several radiation resistant vegetative bacteria were irradiated at 0 and 20 C. Cells irradiated at 40 and 50 C were much more sensitive to radiation. Heating the cultures prior to irradiation lowered the radiation resistance.

Drake, Evans and Niven (65) irradiated survivors of a heat treatment that killed 90 percent of Streptococcus faecium strain HS-5 cells. The survivor curve had less initial lag, but generally paralleled that obtained with unheated cells. Suspensions initially irradiated to 90 percent lethality were slightly more heat sensitive than unirradiated cells. The initial lag phase was again eliminated. Sodium chloride in the suspending medium appeared to sensitize the cells to both heat and irradiation.

Shoesmith (134) demonstrated that viability and pathogenicity were inactivated exponentially at 100 C in Clostridium tetani spores, requiring 15 and 25 min for a tenfold decrease in viable count and pathogenicity. The same loss in viability by gamma rays required 2.6×10^5 rad, while 6.0×10^5 rad were necessary for 90 percent loss in pathogenicity.

Postirradiation heating has been found to enhance radiation lethality or to increase survival. Ehret et al. (111) obtained restoration of a considerable fraction of anaerobically irradiated Bacillus megaterium spores on exposure to higher temperatures after irradiation. Damage was not reversible, however, if anaerobically irradiated spores were exposed to oxygen before being heated. Tallentire and Powers (141) were able to restore Bacillus megaterium spores dried in air by postirradiation heating.

Vacuum-dried spores treated similarly were not restored. According to Tallentire and Davies (104), the probability of postirradiation oxygen-induced lethality for dry Bacillus subtilis spores is greatest at 37, 45, and 50 C.

Harris and Whitefield (145) examined the influences of postirradiation temperatures in the range 8-37 C on the viability of Escherichia coli finally incubated at 37 C. X-irradiated cells had greatest survival if they were held for 24 hr at 15 C before viability was determined at 37 C. Dharkar (132) observed that gamma irradiation sensitized organisms in orange juice to subsequent heat treatment. The sterilizing radiation dose was 8×10^5 rad, but half this dose sufficed if the juice was incubated for 15 min at 50 C following irradiation.

Morgan and Reed (125) showed that gamma irradiation increased the susceptibility of thermophilic anaerobe NCA 3814 to subsequent heating at 240 F (116 C). Prior heating did not increase the radiation sensitivity of Bacillus coagulans and B. stearothermophilus.

Licciardello (128) obtained significantly greater destruction of Salmonella typhimurium when radiation (20,000 - 125,000 rad) and heat were applied simultaneously than when they were applied consecutively. The lethal effect was greatest at irradiation temperatures above 110 F (43 C). In another report, Licciardello (186) noted that a complementary effect occurs only when irradiation

precedes heat treatment. Spores differ in the degree to which radiation sensitizes them to heat. Several species are sensitized to a lesser extent when they are irradiated in complex organic media than in buffer. When Salmonella typhimurium was heated simultaneously with irradiation, there were far fewer survivors than when heating followed irradiation.

According to Grecz, Walker and Anellis (179), 0.4 Mrad gamma rays significantly reduced Clostridium sporogenes PA 3679 spore resistance to conventional heat and to a greater degree to microwave heat. Radiation resistance was only slightly affected if either mode of heating preceded irradiation.

Hollaender (88) noted that cells are more sensitive to heat after X-rays, but reactivation of an Escherichia coli strain occurred if incubation was at 40 C. Infrared around 10,000 A given before X-irradiation will increase the production of mutations in irradiated Aspergillus terreus (88).

Licciardello (127) found that organic matter and oxygen must be absent for vitamin K_5 to sensitize Salmonella to gamma radiation. Moderate temperatures did not enhance radiosensitization by K_5.

Gaseous Atmosphere.

Oxygen. Oxygen sensitizes virtually all biological systems and many chemical systems to inactivation or alteration by ionizing

radiations. The enhanced response has been termed the 'oxygen effect'. Cells are generally more radioresistant in the absence of oxygen (anoxia). Many agents which modify radiation damage do not act per se, but rather by bringing about a change in the oxygen concentration in the vicinity of the irradiated cell. Nitrogen gas is inert, and is generally bubbled through the suspension to achieve anoxia.

Oxygen present during irradiation greatly enhances the lethal action of ionizing radiation toward dry microorganisms (93, 99, 100, 103, 111, 120, 141, 171, 187) and also lessens survival of organisms in suspension during irradiation (72, 88, 112, 135, 140, 170). Bachofer and Pottinger (91), however, discovered that oxygen protects T_1 bacteriophage against both irradiation and hydrogen peroxide. Neither oxygen, air, nor nitrogen influenced survival of organisms in irradiated orange juice (132), presumably because of the presence of protective substances. Oxygen continues to cause lethal damage if present after irradiation (99, 103, 104, 110). Tallentire and Davies (103) discovered that water prevents the latent damage attributed to oxygen.

Nitric Oxide. Nitric oxide is also active in modifying radiation injury. Organisms exposed to nitric oxide in advance of radiation (102) displayed enhanced radiosensitivity. Tallentire and Dickinson

(105) demonstrated that dry Bacillus subtilis spores irradiated in vacuum displayed a postirradiation oxygen effect. Nitric oxide admitted between exposures to oxygen stopped further development of the oxygen effect.

Powers, Kaleta and Webb (171) observed that dry spores of Bacillus megaterium were protected when nitric oxide was present during irradiation or in the first few hours after irradiation in nitrogen. Ehret et al. (111) also found nitric oxide protected anaerobically irradiated Bacillus megaterium spores. Thermorestoration of a considerable fraction of these spores occurred on exposure to higher temperatures after irradiation. On the other hand, damage was not reversible if anaerobically irradiated spores were exposed to oxygen before they were heated.

Howard-Flanders and Jockey (140) found nitric oxide and oxygen to be equivalent in doubling the radiosensitivity of Shigella sonnei during irradiation. Lynch and Howard-Flanders (142) established that pretreatment of this species with nitric oxide in anoxia, or with N-ethylmaleimide (NEM), a sulfhydryl scavenger, rendered the organism more sensitive to anoxic irradiation. Their data indicated that lethality was greatest when sulfhydryl groups were depleted by NEM and irradiation took place in oxygen.

Ultraviolet Radiation. Gol'dat and Alikhanyan (171) found that if spores of Streptomyces aureofaciens were exposed to X-rays after

ultraviolet treatment the number of survivors was considerably higher than expected considering the two irradiation acting separately. Small ultraviolet radiation doses were also reported to be protective to Saccharomyces cerevisiae according to Elkind and Sutton (153, 171). The protective action was demonstrated for yeast exposed to ultraviolet either before or after X-rays. It is evident that ultraviolet and ionizing radiation in combination should not be considered a satisfactory sterilization procedure.

Visible Light. Elkind and Sutton (153, 171) found that visible light reverses the ultraviolet protection of X-ray lethality as well as ultraviolet lethality. Savage, Howell and Clark (147) noted that Nocardia corallina and Staphylococcus aureus could be photoprotected against X-ray inactivation. Staphylococcus aureus was protected if incubated at 37 C but not at 29 C. The physiological state of the organism and the culture medium were shown to influence the degree of photoprotection. These results suggest that radiation experiments should be performed in the dark for maximal lethality. If possible, the irradiated objects should be maintained in the dark.

Water. Tallentire, Dickinson and Collett (110) determined that the lethal efficiency of radiation increases by about 35 percent in changing from the driest spore state to a condition of 100 percent relative humidity at room temperature. They considered water vapor

partial pressure a factor influencing the sterilization efficiency of a given dose of radiation. Very dry Aspergillus terreus spores are notably resistant to X-rays (88).

Davis, Silverman and Keller (120) and Silverman, Davis and Keller (187) found that spores of several species maintained in ultrahigh vacuum for several days were more sensitive to gamma radiation when irradiated in air than in vacuum. Desiccant-dried spores irradiated in air were more resistant than the ultradry spores exposed to air during irradiation and were as resistant as ultradry spores irradiated in vacuum.

Radiation and Spacecraft Sterilization. The radiations in space would not sterilize a spacecraft in flight because of penetration and dose limitations (169). Sterilizing doses of radiation have been reported (6, 7) to degrade spacecraft components to the same degree as dry heat.

There are several limitations on the application of radiation in spacecraft sterilization. Gamma ray intensity is not uniform throughout a radiation field because of the spatial orientation of the Cobalt-60 radiation source. A dosimetry diagram must be prepared of the space utilized for radiation exposure. The radiation intensity decreases with time since the half-life of Cobalt-60 is 5.3 years. These factors relate to the time required for the subject to receive the desired radiation dose. The estimated

dose can be obtained as an average based on the intensities in planes perpendicular to the radiation source. The dose is therefore not precisely determined, but is assumed uniform for a small homogeneous subject.

Hall and Bruch (22) noted that only heat and radiation achieve both surface and internal sterilization. Dry heat was considered the preferred agent. The order in which heat and radiation are applied influences microbial survival as does the atmosphere during and after irradiation. Bruch (168) had suggested that dry heat and radiation in combination may prove feasible for sterilizing components degraded by sterilizing doses of each agent.

The internal construction of all components to be irradiated must be known to the personnel responsible for operating the radiation facility. Metallic parts of the irradiated objects will absorb and scatter incident radiation. Metallic elements would shield material not in the direct path of the rays so that part of the object will not receive the desired dose. Since radiation effects are not subject to interference by interruptions in dose delivery (113), it may be practicable to rotate or turn the component or module to expose all areas likely to be shadowed in any orientation. The inhomogeneity of the radiation field would impose a limit on the size module to be treated.

Radiation effects in materials are not generalized, but are a function of their chemical composition. One cannot properly relate radiation data obtained with model systems from which organisms are easily retrieved for viability studies to the essentially insoluble matrix of an electronic component. Formation and fixation of free radicals important in killing microorganisms will depend on the material harboring the microbes. Component simulants would therefore be useful in heat studies, but of questionable value in a radiation program. A radiation dose known to sterilize a model system might not be adequate to sterilize an electronic device which by chance included a radiation protective chemical intended to serve another function.

The gaseous atmosphere in the radiation chamber can be expected to influence radiation-induced chemical reactions in the surface of impermeable plastics and resins such as would be found in spacecraft components. At appropriate doses, reactions would include decomposition, volatilization, or a transformation which can interfere with the insulating or protective function of the material (169). If air is present, ozone and oxides of nitrogen will be produced as a consequence of irradiation. These gases may then react with the item being irradiated.

The atmosphere would therefore be most significant insofar as material deterioration is concerned, and secondly as a dose

reducing factor. Should it be feasible to apply radiation and heat below the threshold levels for material damage, and it is known that oxygen is not deleterious, it would be desirable to specify an air or oxygen atmosphere to introduce the oxygen effect on the surface contamination present on the irradiated object.

INACTIVATION BY ULTRAVIOLET RADIATION

Bactericidal ultraviolet lamps radiate about 90 percent of their energy at 2537 A, a wavelength near 2650 A, the maximum for lethal activity. Ultraviolet penetrates only slightly below the surface of a liquid, and is primarily used to restrict microbial growth on surfaces and to sterilize air. Other than the indirect action of ozone produced in small quantities by these lamps, any shielding makes ultraviolet essentially ineffective unless a reflective surface is interposed.

Visible light of certain wavelengths applied after ultraviolet exposure can reverse the apparent ultraviolet inactivation. There is also evidence (157) that visible light can partially protect organisms subsequently exposed to ultraviolet. Ultraviolet can also increase sensitivity to heat, but this combination would be applicable only to surface sterilization under conditions of limited heat penetration within solids.

Perhaps the main use of ultraviolet would be in spacecraft assembly. Ultraviolet lamps in rooms or isolators used for spacecraft assembly will destroy airborne microorganisms provided they are not contained within dust particles or otherwise shielded. High intensity ultraviolet lamps in air conditioning ducts can destroy 90-99 percent of the organisms passing by the lamps.

Nagy (op. cit.) presented data necessary for calculating the lamp requirements for various size installations.

Federova (148) discussed the ultraviolet resistance of microorganisms in connection with protection which might be afforded by cosmic dust and the possibility of interplanetary transport of microorganisms. The ultraviolet resistance of microorganisms varies over a wide range of ultraviolet energy. Spores of bacteria and fungi are 10-40 times more resistant than vegetative cells. Organisms which produce dark spores are most resistant, but no organisms withstand about 2 X 10^3 erg/cm^2/sec longer than 3-4 min. Inactivation of 99 percent of the organisms in a closed room requires 1-1.5 X 10^6 erg/cm^2. Exposure to visible light prior to ultraviolet increases resistance to ultraviolet. A spore coated with dead cells or within a speck of dust will be somewhat protected because of the low penetration of ultraviolet.

In a recent publication, Nagy (American Industrial Hygiene Association Journal, v. 25, pp. 274-281, 1964) reviewed the properties and biological effects of ultraviolet generators. A useful table lists the energy required at 2537 A for 90 percent inhibition and for complete destruction of over 40 species of bacteria, yeasts, and molds. Aspergillus niger spores, the most resistant organism cited, required 132,000 microwatt-sec/sq cm for total destruction of colony forming capacity. Special lamps

emit a controlled amount of 1849 A radiation. This wavelength dissociates oxygen to produce ozone. Ozone is bactericidal at 0.04 ppm and 60-90 percent relative humidity.

Webb, Clark and Bailey (158) disclosed that large proportions of staphylococci will survive ultraviolet irradiation.

Cowling, Field and Alexander (Official Digest, v. 34, pp. 816-842, 1962) found that organic coatings considered for spacecraft temperature control are less rapidly degraded by ultraviolet radiation in a simulated space environment than in the normal terrestrial atmosphere.

Water Vapor.

Atmospheric water vapor absorbs much of the bactericidal ultraviolet. Most microorganisms are much more sensitive to ultraviolet when irradiated in a dry atmosphere or in vacuum.

Beebe (167) demonstrated that bacterial aerosols irradiated with bactericidal ultraviolet light are protected by water vapor in the atmosphere.

Luckiesh et al. (150) demonstrated that while certain organisms displayed increased resistance to ultraviolet with increasing humidity, others appeared less resistant at high humidities. Airborne mold spores were unaffected by wide variations in humidity. Aspergillus niger spores are notably resistant, requiring 1,800 and 9,000 microwatts/sq cm-min for 50 and 90 percent in-

activation in air, and 1,300 and 3,000 microwatts/sq cm-min for the same inactivation when irradiated on the surface of a culture medium. The high resistance of A. niger spores is attributed to the increase in free radicals in the spore melanin and darkening of melanin by ultraviolet.

Woodward et al. (152) studied the influence of water on ultraviolet sensitivity in several organisms. Neurospora conidia irradiated dry yielded about five times as many colonial mutants as conidia irradiated in water. Conidia were most sensitive to ultraviolet at or below 15 percent relative humidity. Bacteriophage XP4 was especially resistant to ultraviolet at 35 percent relative humidity, but in phage T_3, ultraviolet sensitivity was directly proportional to the degree of hydration.

Oxygen.

Zetterberg (159) could not detect any influence of oxygen on mutagenic or killing action of ultraviolet light exposure on Ophiostoma.

Oxygen protects T_1 bacteriophage against irradiation and hydrogen peroxide inactivation (91). The lethal effect of hydrogen peroxide and ultraviolet acting simultaneously was greater than the activity of each agent acting separately. Oxygen appears to be required for photoreactivation in Bacillus subtilis 168 (164).

Potentiation of Thermal Lethality.

Duggar and Anderson (124) reported that exposure of Saccharomyces cerevisiae to ultraviolet light followed by heat treatment at 50 C is 2-5 times more lethal than if the heat precedes ultraviolet irradiation.

Curran and Evans (76) showed that spores were more susceptible to heat after exposure to ultraviolet light. Bacillus cohaerens, the most heat resistant of three organisms studied, was most readily sensitized to heat by ultraviolet. Wavelengths in the 350-1,600 A range were more effective than 2537 A in inducing heat sensitization at 98 C.

Doudney (155) deduced that RNA and protein synthesis during the initial period of incubation following ultraviolet exposure is necessary for bacterial recovery but detrimental to subsequent recovery.

Photoreactivation.

Jaggar (157) observed that Escherichia coli B cells exposed to light of 3,100 to 6,000 A prior to exposure to low doses of 2537 A ultraviolet light were partially protected from ultraviolet.

Ultraviolet-inactivated Endamoeba histolytica achieved maximum photoreactivation with a 45 min treatment with visible light (156). Kelner (164) described experiments which indicated that Bacillus subtilis 168 requires oxygen for photoreactivation

after ultraviolet irradiation.

Modification of Ultraviolet Resistance.

According to Webb and Clark (151), successive ultraviolet irradiation of Nocardia corallina coccoids produced a significant increase in sensitivity.

Adler and Hardigree (165) found that mutation at the lon locus in Escherichia coli K-12 results in enhanced radiation sensitivity and several morphological changes. Another series of mutants (166) are more sensitive than the parent strain to ultraviolet, and differ from the lon mutant in several characters.

Nonlethal exposure to ultraviolet is known to produce significant numbers of mutants in some organisms. Mutational synergism has been shown by Shankel and Coupe (160) to greatly increase the number of mutants in Escherichia coli irradiated sublethally and then allowed to develop in the presence of caffeine. Alkaline conditions and incubation at 37 or 42 C favored the expression of mutants.

Ultraviolet resistance has been transferred in mating strains of Escherichia coli (161)

Payne and Campbell (162) determined that iron can protect Micrococcus violagabriellae from ultraviolet damage and possibly permit recovery from such damage. Subsequently (163), they obtained

evidence suggesting that a cytochrome-linked electron transfer system was ultraviolet sensitive.

A method for detecting ultraviolet damage producing respiratory deficiency was described by Pittman and Roshanmanesh (154).

Chemicals.

Bruni (149) noted that ultraviolet activated the oligodynamic action of silver.

Assessment of absolute lethality in any treatment involving ultraviolet should include a brief exposure of the material or organisms to visible light so that repairable cell damage can be detected.

THERMAL RESISTANCE, DESICCATION, AND AEROSOLIZATION

The thermal resistance of a microorganism is influenced by all environmental conditions it encounters in its life history. Spores are much more resistant than vegetative cells. Some of the better known factors which play important roles in the apparent heat resistance of a culture include the composition of the sporulation medium, temperature during sporulation, the species and particular strain, the age of the spores, degree of hydration, the spore carrier, the gaseous atmosphere during heat resistance studies in the dry state, the composition of the heating menstruum surrounding spores heated in liquid, the requirement for activation by heat or chemicals to trigger germination and break dormancy, the composition of the culture medium in viability studies, and the incubation temperature. Several of these determinants of apparent heat resistance will be considered briefly. Sensitization of organisms to heat by ionizing and ultraviolet radiations is treated elsewhere in this report.

Thermal Resistance in Dry and Moist Environments.

Bruch (168) reviewed the biological and physical factors in dry heat sterilization. He cited the enhancing action of vacuum against organisms exposed on surfaces, differences in the order of

sensitivity of spores in several hot gaseous atmospheres, and the influence of moisture in the hot gases on spore resistance. He observed that solids are difficult to sterilize by dry heat. The presence of plastics in space probes will make sterilization difficult.

Jaffe (169) noted that many spacecraft components are damaged in 24 hours at 135 C. Heat sterilization might be useful in flight, but it would be difficult to heat a spacecraft uniformly and avoid interference with instrument calibration.

Bělehrádek (62) compiled a table of temperature coefficients of the rate of injury by heat for a number of spores and bacteria. Over the temperature range 120 - 135 C, dry Bacillus anthracis spores had a Q_{10} value of 2.9.

Koesterer and Bruch (69) compared the resistance of dry spores of Bacillus subtilis var. niger and B. stearothermophilus strain 1518 to destruction by moist and dry heat. Sterilization of 1-10 X 10^5 spores was accomplished in less than 5 min for B. subtilis var. niger at 110 C, and in 20 min at 121 C for B. stearothermophilus. B. subtilis var. niger spores were 3.5 to 5 times more resistant to dry heat than B. stearothermophilus spores. The times required to kill 90 percent of the spores at 121 C were 47.5 and 14.3 min, and 1.6 and 0.3 min at 160 C respectively, for B. subtilis var. niger and B. stearothermophilus.

Bruch, Koesterer and Bruch (70) considered the dry heat resistance of spores on several carriers. This research was aimed at development of dry heat cycles for sterilizing spacecraft components. In experiments at 125 C, the presence of soil, sand, and vermiculite increased the apparent heat resistance well beyond the resistance of spores on paper strips or in test tubes.

Pheil, Nicholas, and Pflug (77) showed that the nature of the dry gas surrounding cells during heating influences the destruction rate of Bacillus subtilis spores. Helium and nitrogen were less lethal than oxygen, air and carbon dioxide.

Wende and Burdon (66) investigated the dry heat sterilization of bonemeal contaminated with Bacillus anthracis spores. Samples weighing 25 g were sterilized after 60 min at 160 C while 40 min sufficed for 10 g samples. Morphological and toxigenic variants increased with the degree of heat exposure.

Zampach (64) sterilized plastic bands of a microbial air sampling device with dry heat at 140 C in 3 hr. A revolving drum was sterilized by means of an antiseptic such as mercury phenylborate.

Davis, et al. (71, 81, 187) observed that spores varied in resistance to moderate temperatures in ultrahigh vacuum. Viable Aspergillus niger spores were recovered after 4-5 days in ultrahigh vacuum at 107 C, but none were viable at 120 C. B. subtilis var.

niger spores did not survive 90 C in vacuum, but significant numbers were recovered in experiments conducted for 5 and 7 days at 90 C and atmospheric pressure.

Henderson and Dinning (73) obtained 0.1-1 percent survival of dry Aspergillus niger spores after 3 hr at 100 C. When the spores were suspended in water, only 0.00001 percent were recovered after 1 hr at 100 C. Spores in deuterium oxide were 1,000 to 10,000 times more resistant than spores in water.

An exceptionally heat resistant Bacillus subtilis was isolated by Iyer and Bhat (63) from Bombay air. Survivors were observed after 60 min at 120 C in aqueous suspensions containing 10^5 spores.

Sporulation Environment and Thermal Resistance.

The thermal resistance of Bacillus coagulans spores was found by Lechowich and Ordal (67) to increase with increased sporulation temperature. The cation contents of these spore crops were similar, but the dipicolinic acid content decreased as the sporulation temperature increased.

Tallentire and Chiori (109) were able to double heat resistance in Bacillus megaterium spores produced in an Mn-Ca-Mg medium instead of an Mg-Fe medium.

Spore Activation and Determination of Viability.

The environmental and nutritional requirements for spore germination are poorly defined. Heating spores at temperatures

usually considered lethal for vegetative cells is often necessary to achieve maximal colony counts under 'normal' incubation conditions. These 'heat shock' temperature-time relationships are quite arbitrary, and there is no assurance of quantitative recovery of all viable spores. It is possible that stresses imposed on an organism undergoing sublethal heating or sublethal irradiation alter the normal metabolic and growth patterns so that although the spore is viable, the environment is not conducive for proliferation.

An organism exposed to a combined treatment with two agents might be more refractory than under a single environmental stress. Other sections of the report illustrate the reversibility of apparent lethality by agents seemingly unrelated to one or more damaging physical or chemical sterilant. Several citations serve to illustrate the problems encountered in assessing microbial survival.

Segner (74) obtained a tenfold increase in viable count when Bacillus stearothermophilus spores were heated to 120 C and cooled, compared with counts after heat activation at 100 C.

Graikoski and Kempe (86) found that activation of dormant Clostridium botulinum Type E spores heated in neutral buffer occurred during 40-60 min heating at 70 and 75 C although significant

numbers of spores were killed in 20 min. Survivors were obtained after heating for 60 min at 90 C.

Thermal resistance of spores heated in suspension can depend on the buffer composition and pH (80).

The composition of culture media for heat-treated bacteria can influence the apparent survival. Low Co^{++} concentrations increase survival of sublethally heated nonsporulating bacteria five-to-tenfold, while Cu^{++} is inhibitory (82).

The highest growth temperatures confirmed for microorganisms are 72-75 C (85).

A mesophile resembling Bacillus megaterium was able to grow at 65 C in association with a thermophile resembling B. stearothermophilus (83). Johnson and Chan (78) found that Arthrobacter globiformis, a soil organism that grows well at 25 C, would grow at 30 and 37 C only in the presence of another organism which could grow at these temperatures. It would be interesting to determine whether presumably sterile surfaces do indeed harbor viable organisms which require commensal association to grow at usual incubation temperatures.

The longevity of anaerobic sporeformers was reported by Haynes and Rhodes (56) to exceed 13 years in lyophil preparations and 20 years in soil.

Microbial Aerosols.

Levin and Cabelli (53) reported that germination of Bacillus subtilis var. niger spores occurred in the processes of aerosolization and collection in liquid impingers. Germination was dependent on oxygen and independent of exogenous nutrients. The spores collected had increased heat susceptibility.

Cabelli (51) and Hayes and Cabelli (58) attributed loss in Pasteurella tularensis cell viability to the altered permeability resulting from aerosolization and rehydration.

Hatch and Dimmick (57) related the death rate of microorganisms aerosolized at various relative humidities to an inbalance in metabolic function prior to aerosolization.

Greene (79) exposed aerosols of lyophilized Serratia marcescens and Bacillus subtilis var. niger spores in a wind tunnel to temperatures ranging from 75-200 C for about one sec. S. marcescens required exposures of 150 C for 1.68 sec to 170 C for 0.5 sec for 99.9 percent kill. Exposures of 160 C for 1.68 sec to 200 C for 0.5 sec were necessary for 50 percent inactivation of B. subtilis var. niger spores. Protective effects were noted when the lyophilized suspensions were coated with colloidal silica powder before aerosolization.

CHEMICAL STERILIZATION

The sterilizing efficiency of chemical sterilants depends to a great extent on environmental factors. Opfell (27) has given careful consideration to the application of chemicals in spacecraft sterilization. He noted that chemicals should be considered only if physical agents such as heat and radiation are not compatible with spacecraft components. Factors limiting sterilant efficiency were discussed. Recommendations were made for improving the reliability of sterilization processes.

Chemicals can readily pasteurize soil (30), but sterilization of soil is difficult to achieve. Formaldehyde is the best agent other than steam for retaining soil properties. A formaldehyde-steam sterilization process has been described (35).

Gaseous Sterilants.

A useful review of ethylene oxide sterilization procedures was compiled by Stierli, Reed and Billick (43). Bruch (Annual Review of Microbiology, v. 15, pp. 245-262, 1961) reviewed biological action of gaseous sterilants and discussed numerous medical, agricultural and industrial applications of gaseous sterilization.

Jaffe (169) observed that ethylene oxide gas is compatible with most spacecraft components, but penetration is limited. On relating population reduction factors with ethylene oxide kill

curves, Jaffe concluded that there is insufficient evidence to show that ethylene oxide can produce high probabilities of sterility in spacecraft applications.

Tessler (40) found that several plastics are damaged by gas sterilization, and recommended that the stability of plastics be determined under conditions which would indicate gross damage.

Pan and Gast (39) described a procedure for determining the antimicrobial activity of gases. Their technique may be suitable for confirming the reliability of a gas sterilization process considered for spacecraft assembly, and intermediate or terminal sterilization.

A method for sterilizing and protecting surgical instruments from recontamination was developed by Montgomery and Morelli (49) as a space-oriented technique. The instruments are dipped into an ethylene oxide-liquid plastic mixture which subsequently forms a solid polymer film retaining residual ethylene oxide.

Opfell et al. (42) concluded that convection was necessary as well as diffusion for a gas to reach sterilizing levels at a remote surface in a confined space.

Humidity and Temperature. The interplay of humidity and temperature and the concentration of the gaseous sterilant are prime considerations in any gaseous sterilization process. These parameters are important for single gases and for mixtures of gases such as ethylene oxide

and propylene oxide or ethylene oxide and methylene bromide (37, 38).

Bomar (41) showed that dried Bacillus subtilis spores stored for 8-14 days had far greater resistance to ethylene oxide than 0-4 day-old preparations.

El-Bisi and his associates (45-47) studied the influence of moisture, humidity and temperature on ethylene oxide sterilization.

Brown and Fuerst (44) demonstrated that more than 24 hours were required at 37 C to kill large inocula of Aerobacter aerogenes, Escherichia coli, Bacillus megaterium bacteriophage, and a yeast in 1 percent ethylene oxide at 60-80 percent relative humidity.

Ionizing Radiation. Phillips (34) reported that the lethality of several gas phase and liquid phase germicides is enhanced by ionizing radiation. A potentially useful sterilizing treatment combined ethylene oxide and 100,000 rad or less ionizing radiation.

Liquid Sterilants.

Jaffe (169) discussed limitations of liquid sterilants, and cited a number of factors which influence their effectiveness. A discussion of spacecraft assembly techniques considered the chance of contamination under clean room conditions.

Willard and Alexander (48) incorporated formaldehyde in a Surveyor thermal control coating which cannot be gas sterilized. This coating, however, did not always sterilize the surface in

contact.

Beta-propiolactone (BPL) is an efficient liquid and vapor phase sterilant (24-26). The chemical will corrode certain metals and plastics (25) but hydrolyzes in water to harmless substances. LoGrippo and Hartman (26) found less BPL and ultraviolet were required together for virucidal action than each agent individually.

Peracetic acid is a useful surface sterilant (29) that has been used in conjunction with anionic detergent. Wuhrmann and Meyrath (36) observed the superiority of ozone over chlorine dioxide and hypochlorite as germicides. The sporicidal action of alcohol is heightened by any of several acids and bases (32). Several reports (27, 33, 145) note the importance of temperature control in the use of germicides.

Miscellaneous Chemical Applications.

An electric current was found by Rechmenskii (59) to activate the sterilizing action of mercury compounds on Bacillus anthracis spores so that less time or a lower concentration of the agent was required.

Fungi in cooling tower wood were killed in 2 hr at 145-160 F (63-71 C) (68). A phenolic bactericide was then crystallized on the sterilized wood to retard future attack.

ELECTRIC AND MAGNETIC FIELDS

Electric Fields. The various radiations in the electromagnetic spectrum affect living organisms quite differently. The energy is proportional to the frequency, and is sufficient to produce ionization at the frequency of X-rays, and the visible portions of the spectrum. The energy is much too low to produce ionization in the lower radiofrequency megacycle and microwave ranges. At these frequencies the primary effect observed is heating attributed to the resistance of the electromagnetic wave passing through a "lossy" dielectric. Radiofrequency absorbing material converts the absorbed energy into heat. There is evidence for nonthermal effects of radio waves which are as yet undefined. It is possible, for example, to achieve gross killing and mutation effects with equipment which pulses the radio waves so that no measurable temperature change is detected. These experiments are especially interesting because the energy is applied for total exposures of seconds or minutes at low power.

There is a considerable literature on biological effects of radiofrequency energy, but it is difficult to reproduce experiments in several laboratories because of the variables associated with the equipment and experimental conditions. Radiofrequency equip-

ment must be designed for a particular application, and is generally not available as an off-the-shelf commodity. There is good evidence that killing of microorganisms is frequency specific within a narrow band. Thus conditions shown to kill all Salmonella senftenberg in cocoanut meat do not injure bacterial spores.

Ark and Parry (172) reviewed the pre-1940 literature on biological effects of high frequency oscillating fields. They called attention to the specific frequency requirements for killing various bacteria and fungi. Erwinia carotovora suspended in an electrolyte was killed rapidly at 10.4 meters but was not killed in the dry state at this wavelength. Lethal effects of a wavelength of 5.6 meters against several fungi began when the temperature reached 30-40 C. This temperature range was not lethal in the absence of a field.

Nyrop (173) summarized the results of a series of investigations in which the specific effects of electric field with minimal heating were explored. These experiments were at 20 mc. Bacteria and virus were killed in exposures of 10 sec or less. Unfortunately, Nyrop died without preparing detailed reports, and none of his experiments will be published other than the single citation.

Although penetration and scattering is a function of each

frequency, Kosikowsky, Herrington, and Dahlberg (174) raised the temperature of a 1.3 lb block of cheese to 117-155 F (47-68 C) within 1.5-2.7 min by means of an oscillator operating at 150 mc at a possible power output of 750 w. The bacterial count was greatly reduced by this treatment.

Jacobs, Thornley, and Maurice (175) recognized the variables associated with any application of megacycle range rf as a bactericidal agent, but did not exclude the possibility that marked effects might be obtained at highly critical frequencies. These workers considered the bacterial cell as a complex of molecules bearing unequally distributed electrical charges. A rapidly alternating electric field of high potential gradient might disrupt an essential molecule at a weak bond. Limitations imposed by the frequencies and power in their experiments, the electrode arrangement, and failure to induce high mortalities, led this group to conclude that it is unlikely that this technique will have any practical application in sterilization other than providing uniform heating.

King (177) showed that microwave heating at 2450 mc followed by ionizing irradiation inactivated proteolytic enzymes in meat, yielding a product of good quality. Radiation sterilization processes ordinarily require considerably higher doses for enzyme

inactivation than are required for microbial inactivation. The food product may then be organoleptically unacceptable.

Grecz, Walker, and Anellis (179) found that microwave heating at 2450 mc in a constant temperature device was consistently much more lethal to Clostridium sporogenes PA 3679 spores in buffer at 85, 95, and 100 C than conventional heating. Microwave treatment of dry spores did not influence viability. A dose of 0.4 Mrad gamma rays significantly decreased spore resistance to heat and to a greater degree to microwave heat. Radiation resistance was only slightly affected if microwave or conventional heat preceded the irradiation.

Heinmets and Herschman (178) indicated that it is difficult to obtain uniform fields in experiments with combined magnetic and electric fields. They postulated that combined fields would not necessarily kill cells directly, but would cause abnormal cell growth which might lead to cell death. The greatest activity of combined fields would occur when electric and magnetic fields of a travelling wave are in phase and perpendicular to each other. Dielectric heating of the medium or the object occurs. These views relate to particles in liquid.

The 20-30 mc range has been determined by John Heller and his associates at the New England Institute for Medical Research

Ridgefield, Connecticut to be bactericidal. Pulsed rf experiments performed at Ridgefield by personnel of the Research Laboratories, Wilmot Castle Company, showed that bacteria were killed on a variety of objects but none of the experimental conditions accomplished sterilization. The power requirements for rf studies are quite low, but a complex interaction of several variables presents the greatest barrier limiting an orderly experimental program. These variables include frequency, field strength (voltage), pulse width (if not continuous wave), repetition rate, and total time in the field. It is likely that a system in which rf and moderate heat were applied simultaneously would be most effective since slight heating seems to enhance the rf effect. A continuous wave process rather than a pulsed system might be useful if heating can be tolerated.

Heller has demonstrated (unpublished) that rf sterilized semiconductors which had microbial contamination without affecting performance of the component. In any extension of this work, however, it will be necessary to manipulate the exposure conditions to determine the appropriate system which will avoid any possibility of damage due to excessive rf energy. Perhaps the primary limitation of rf in any spacecraft application would be the metallic elements in electronic devices. Metals provide total shielding to rf so that the field becomes distorted and the metal heats up. Most

plastics are transparent to rf and would be good candidates for an rf study. It should be possible to sterilize certain spacecraft components and subsystems nonthermally with rf prior to final assembly. Transistors and diodes would probably be less reliable after an rf treatment at high field strength. In an extension of this concept, it may be possible to sterilize the spacecraft exterior prior to launch or after returning from an extraterrestrial mission by imposing a symmetrical field around the spacecraft.

Magnetic Fields. Magnetic fields do not appear to be useful in sterilization. Growth inhibition has been noted in cultures maintained in magnetic fields, but several species have been unaffected by conditions which influence the growth rate of other organisms. Exposures of long duration to strong fields seem necessary to demonstrate significant inhibition. All reports cited concern cultures in suspension. The response of organisms in anhydrous environments or under conditions in which the cells are immobilized does not appear to have been investigated. It is probable that inductive effects of magnetic fields would adversely affect diodes and other components.

Halpern and Greene (61) did not find any magnetic field effect on HeLa cells. Beischer (180) employed a much stronger field (140,000 Oe) but could not detect mutants in Neurospora

crassa conidia in a 2-hr exposure. The light emission from Photobacterium fischeri was not changed after 1-hr in this field.

Gerencser, Barnothy and Barnothy (181) studied bacterial growth in magnetic fields. Serratia marcescens cultures in a field averaging 15,000 Oe had the same growth rate and cell numbers as control cultures for up to 6 hr. The magnet cultures then increased at a slower rate for several hours but then increased at a greater rate than the control so that after 10 hr the magnet culture surpassed the control.. Staphylococcus aureus magnet cultures had an early period of more rapid growth than the control. Growth inhibition then occurred for several hours, but magnet and control cultures were equivalent at the 9th hr.

Hedrick (182) also observed growth inhibition in magnetic field cultures. Staphylococcus aureus exposed to a 14,000 Oe homogeneous field and control cultures were similar for 15 hr. Inhibition of growth in the field was noted at the 16th hr and continued until the end of the 24 hr exposure when the plate count was about one-fifth that of the control. Inhibition did not occur if the homogeneous field was interrupted hourly for 3 sec. Neither Sarcina lutea nor Escherichia coli showed cell population differences in similar experiments.

ULTRASONICS

Ultrasonic energy is ordinarily transferred through a liquid to the object being treated. An acoustic wave of high intensity is produced at frequencies of 20,000 and 800,000 cps at submicron amplitudes in several commercial sonicators. Violent pressure changes in the liquid disrupt cells and break molecular bonds.

Although sonication is not useful for internal treatment of solid objects, Ney (122) and Dharkar (133) found that sonication enhanced the killing effect of ionizing radiation even under sonication conditions which were not lethal. Licciardello (186) noted that ionizing radiation has been coupled with ultrasonic energy in attempting to improve the quality of radiation sterilized foods by lowering the radiation dose. Heat and radiation seemed to be a more promising system.

Dalzell et al. (183) found Micrococcus pyogenes var. aureus to be more resistant to sonication than Escherichia coli. Lethal effects were independent of temperature in the range 15-45 C.

Wilson and Curtis (184) described ultrasonic radiation effects on yeast and mammalian tissues.

Berliner and La Rochelle (185) could not detect cell damage in the luminescent fungus Armillaria mellea after 1 sec to 10 min

exposure to 20,000 cps but light emission increased up to 400 percent in several exposures.

Small modules or components which are sensitive to heat, radiation, or gaseous sterilants could be surface sterilized in a short time by ultrasonic energy. It is likely that mild sonication would promote the efficiency of a liquid sterilant so that surface sterilization time could be reduced. A brief research study would be necessary to confirm this view. A system utilizing ultrasonics and a germicide might prove acceptable for sterilizing the exterior of a quarantined spacecraft. The higher frequencies are probably most efficient. Sonication may be helpful in destroying fecal microorganisms in a life support system involving reclamation of water or nutrients in feces (12).

KEY TO TOPICAL INTERRELATIONS AMONG ABSTRACT ENTRIES

A cross-index has been prepared to simplify selection of abstract entries which concern combined methods of sterilization. The index consists of a chart in which the abstract entries listed at the intersection of horizontal and vertical columns relate to both topics. Entries which include significant information on a single topic may be found at the intersection of columns headed by the topic. This key will supplement the subject index.

The topics included in this key are:

Chemicals
Cold
Desiccation
Electric Fields
Gas Sterilization
Gaseous Atmosphere
Heat
Ionizing Radiations
Magnetic Fields
Shock
Spacecraft and Equipment
Steam
Ultrasonics
Ultraviolet Radiation
Vacuum
Visible Light

1

KEY TO TOPICAL INTERRELATIONS

AMONG ABSTRACT ENTRIES

	Chemicals	Cold	Desiccation	Electric Fields	Gas Sterilization	Gaseous Atmosphere	Heat	Ionizing Radiations
Chemicals	23 24 25 26 27 28 29 30 31 32 33 35 42	33 52	51 53 57 58	59 177	35 37 38 39 40 41 42 43 44 45 46 47		33 50 55 67 73 82 83 85 109 145	24 30 32 101 102 107 114 115 120 127 129 130 131 136 137 138 139 140 142 143 165 171 177 178
Cold	33 52	14	51 71					101 107 114 115 170 171 173 178
Desiccation	33 51 53 57 58	51 71	51				71 79 168 187	32 99 100 101 104 103 111 120 133 171 187
Electric Fields	59 177			172 173 174 175 179			172 173 174 175 179	59
Gas Sterilization	35 37 38 39 40 41 42 43 44 45 46 47							34
Gaseous Atmosphere							77 168	77 87 88 91 93 99 103 104 105 110 111 112 120 127 132 135 140 141 142 170 171 176 186 187
Heat	33 50 55 67 73 82 83 85 109 145		71 79 168 187	172 173 174 175 177 179	43 47	77 168	62 62 66 69 70 73 74 78 81 85 86 116 134 148	65 72 75 88 95 98 104 106 109 111 114 125 127 128 132 134 145 168 176 179 186

Ionizing Radiations	Magnetic Fields	Shock	Spacecraft and Equipment	Ultraviolet	Miscellaneous Radiation	Vacuum	Visible Light
94 [illegible] 50 [illegible] 101 102 109 [illegible] [illegible] [illegible] 127 129 [illegible] 131 [illegible] [illegible] 158 [illegible] [illegible] [illegible] [illegible] 165 [illegible] [illegible] [illegible] [illegible]			[illegible] 6 7 [illegible] 27 [illegible] [illegible] [illegible]		[illegible] [illegible] 55 [illegible] [illegible] [illegible] [illegible] [illegible]		
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Ionizing Radiations	[illegible]	Cold	Desiccation	Electric Fields	[illegible] Sterilization	Gaseous Atmospheres	Heat	Ionizing Radiations
[illegible] Fluids				[illegible]				
[illegible]								
[illegible] and Liquids								
Steam	[illegible]							
Ultrasonics							183	122 133
Ultraviolet Radiation	26 149 155 160 162 165 166 171	148	152			91 159 162 165 164 167 171	76 88 124 160 186	151 153 171
Vacuum		71 81 187					71 81 168 187	72 100 103 110 120 141 187
Visible Light								147 153 157

Heat	Ionizing Radiations	Magnetic Fields	Shock	Spacecraft and Equipment	Accelerations	Ultraviolet Radiation	Vacuum	
55 72 75 88 95 [illegible] 104 [illegible] 10[illegible] 111 [illegible] 125 127 128 132 [illegible] 145 [illegible] 170 173 [illegible]	89 90 [illegible] 96 97 101 10[illegible] [illegible] 115 11[illegible] 117 11[illegible] [illegible]19 121 125 129 134 [illegible] 146 151			2 [illegible] [illegible] 52 160 161	122 158	151 152 171	72 100 10[illegible] 120 120 141 [illegible]	[illegible]
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76 88 124 180 186	151 153 171			169		101 118 144 146 148 150 151 152 158 161 183	148	148 1 157 1
71 81 168 187	72 100 103 110 120 141 187					148		
	147 153 157				183	148 153 154 156 157 [illegible] 171		

Ionizing Radiations	Magnetic Fields	Shock	Spacecraft and Equipment	Acoustics	Ultraviolet Radiation	Vacuum	Visible Light
90 94 96 97 10[illegible] 115 11[illegible] 116 119 121 125 134 145 146 15[illegible]			2 [illegible] 100 [illegible]	132 133	15[illegible] 152 172	72 100 10[illegible] 120 141 [illegible]	[illegible] 151 157
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[illegible] 165	122	[illegible]	[illegible]	133			
122 133			185	184			163
151 153 171			169		101 118 144 146 148 150 151 152 158 161 183	148	148 153 154 156 157 164 171
2 100 103 110 0 141 187					148		
147 153 157				183	148 153 154 156 157 [illegible] 171		

1. NEWS OF SCIENCE; DEVELOPMENT OF INTERNATIONAL EFFORTS TO AVOID CONTAMINATION OF EXTRATERRESTRIAL BODIES

Science, v. 128, pp. 887-889, 1958

The National Academy of Sciences of the United States is deeply concerned with the dangers of contaminating extra-terrestrial bodies now that space exploration is a more immediate possibility. At the urging of the NAS, the International Council of Scientific Unions has established (1958) a Committee on Contamination by Extraterrestrial Exploration (CETEX). CETEX is concerned with contamination of lunar or planetary bodies with microorganisms, radioactivity, chemicals in large amounts, and earthly macromolecules. Therefore, CETEX will endeavour to draft a code of conduct for international space investigations without delay.

2. STERILIZATION OF INTERPLANETARY VEHICLES

Phillips, C. R., Hoffman, R. K.

Science, v. 132, pp. 991-995, 1960

Contamination of the moon and planets by earthly micro-organisms is possible unless stringent sterilization techniques are followed before launching. Contamination of these bodies would be a disaster to future biological studies of extra-terrestrial life and to planetary and earthly ecologies. The extreme conditions of the space environment, the entry and re-entry of the vehicle, are not lethal to all organisms. Techniques are available to insure sterility at the moment of launch. The procedures discussed include, (1) sterilization by heat, radiation and chemicals of the spacecraft components during assembly and (2) terminal sterilization of the completed vehicle prior to launch. The use of ethylene oxide within a plastic covering is recommended for the terminal sterilization. Design of spacecraft and the choice of components should be influenced by sterility requirements and by sterilization techniques presently in use.

3. THE ROLE OF FUNGI IN THE DETERIORATION OF MISSILES AND MISSILE COMPONENTS

Lee, C. B.

Developments in Industrial Microbiology, v. 2, pp. 55-64, New York, Plenum Press, 1961

Fungus growth had been found on missile materials in temporary storage during manufacture, and its presence was considered a source of potential difficulty in the future tactical operation of missiles. Prior to laboratory investigations, conferences were held with personnel at the manufacturing site to acquaint them with the importance of fungi as agents of deterioration and to discuss the inter-relationship of various parts of the missile system and the possible loci of potential fungi deterioration. Five points were considered during testing: (1) Determination of the composition of the materials submitted to test, either by information from the supplier or by laboratory analysis; (2) Precise estimation of fungus utilization of test material; (3) Recommendations for particular performance tests on materials to define actual microbiological utilization of items and concurrent correlation of this information with visual observation of fungus growth;(4) Specific determination of species of degrading fungi; (5) Suggestions

3. Cont'd

and methods to be devised to demonstrate protection of materials against fungi and necessary corrective measures. Tests were carried out in the simulated tropical testing facility at the Detroit Arsenal OTAC, instrumented to simulate the conditions of the tropical rain forest. Materials were tested without pretreatment, exactly as received from the supplier. All materials received an inoculation with a mixed spore suspension of six to ten species of fungi of known degrading ability on particular types of materials. Exceptional growth was noted on elastomer buttons, cable leads containing chromatic materials, asbestos tubing adulterated with cotton, code-colored cable harnesses, silk-wrapped transformers improperly sealed against the tropical atmosphere, and solenoids wrapped in natural textile. Many of the assembled control panels and other instrumentation developed fungi at several loci.

4. STERILIZATION OF COMPONENTS FOR LUNAR SPECTROMETER

Boone, I. U., Rivera, L. T., Anderson, E. C.

1962

In "Biological and Medical Research Group (H-4)
of the Health Division, Annual Report
July 1961, through June 1962", pp. 216-220
Los Alamos Scientific Laboratory of the
University of California
Los Alamos, New Mexico
LAMS-2780
TID-4500 (18th Ed.)

Electronic components were sterilized with ethylene oxide gas in an air-tight plastic enclosure at adjusted (but undisclosed) humidity and sterilization time. B. subtilis var. niger spore strips placed at the inlet and outlet and throughout the chamber were used to determine the efficiency of sterilization following culturing in thioglycollate broth. Bactericidal and bacteriostatic properties of some unsterilizable materials (adhesives, etc.) toward S. aureus were determined. The plastic, adhesives, catalysts, and potting compounds tested were all found to be sterile and in some instances (two activators), bacteriostatic, or bactericidal. The components included Stayfoam, 2 epoxides, styrene glue, Eastman 910 adhesive, 2 activators, Armstrong

4. Cont'd

A-1 adhesive, Catalyst No. 7, Stycast No. 40, SN-3, SN-4, and Silastic RTV. The possibility of bacterial spores surviving in plastic was noted, and it was proposed that radiation sterilization of space vehicles is possible if the sterilization requirement is considered in all stages of design and construction.

5. MOON MAY NOW BE CONTAMINATED

Beller, W.

Missiles and Rockets, August 27, 1962, pp. 24-27

Lunik II and Ranger IV, because of imperfect sterilization, may have already contaminated the moon on impact. Even though the lunar surface may be hostile to organisms, unsterilized fuel and electronic components dropping into crevices may have carried viable microorganisms to subsurface areas capable of preserving spores or vegetative forms. This may seriously hinder biological exploration of the moon.

6. SPACE PROBE STERILIZATION

A Review of Space Research, Publication 1079, Chapter 10, pp. 10-1 - 10-10
National Academy of Sciences-National Research Council: Washington, D. C., 1962

"Absolute" sterility is not an operational concept when applied to spacecraft. However, by strict adherence to proven procedures, the probability that a spacecraft is contaminated with viable organisms can be very low. Thus "certified" sterility is an operational possibility. Four techniques presently available for sterilizing spacecraft are dry heat, ionizing radiation, bactericidal liquids or vapors, and filtration of liquids and gases. More work is required to establish dry thermal death kinetics. On the basis of known data, the dry heat treatment recommended by JPL for sterilization of planetary spacecraft is 135 C for 24 hr while for lunar craft, the current requirement is 125 C for 24 hr. Testing must also be done of components which may be degraded at the "certified" sterilizing temperature. These components may be sterilized by penetrating ionizing radiation. However, sterilizing doses of radiation (10^6 - 10^7 rad) degraded some components to approximately the same degree as dry heat treatment. Ethylene oxide is the most

6. Cont'd

effective method of chemical sterilization for surfaces accessible to a vapor. It often takes less time to achieve sterility with ethylene oxide vapor than with an equivalent heat treatment. Further investigation of ethylene oxide for sterilizing components and spacecraft is needed. Glove box procedures with vapor sterilants may be used for adjustments and repairs to previously sterilized spacecraft. Spacecraft liquids or gases which cannot be heated or chemically treated may be sterilized by filtration. New filter assemblies must be designed for each application of the filtration process on the spacecraft.

The JPL program of spacecraft sterilization has achieved substantial technological advances, has developed rapport between biologists and spacecraft engineers, and has identified problem areas for future study. Optimum sterilization will not be achieved on lunar spacecraft, but is possible and necessary for the Mars landing. A more stringent policy for the sterilization of manned landings is urgently needed. It is also urgent that biological experiments be given first priority in all lunar and planetary programs before a high degree of contamination is introduced. Mars should be made a biological preserve. A Sterility Control Group, equipped with internationally agreed-upon policies should be established

6. Cont'd

within all organizations responsible for planetary missions.

7. REVIEW OF THE NASA-JPL SPACECRAFT PROGRAM, Appendix III

Space probe sterilization

Hobby, G. L.

A Review of Space Research, Publication 1079, Chapter 10

pp. 10-25 - 10-36

National Academy of Sciences-National Research Council:

Washington, D. C., 1962

Considerable differences exist between sterilization requirements for lunar missions and those for planetary programs. JPL was made responsible in 1960 for technical implementation of the spacecraft sterilization program. JPL attempted to follow the policy set forth by the NASA Administrator and the Space Science Board. However, reliability of the Ranger spacecraft and its components was given higher priority than its sterility. Dry heat and radiation have been used to insure internal sterilization of electronic components and plastic materials since it is assumed such agents would be effective against spores, virus and vegetative microorganisms. Both heat and radiation are detrimental to spacecraft materials. To minimize degradation of components, a sterilizing temperature of 125 C for 24 hr was specified by JPL. This specification was verified by Bruch. Radiation did not appear promising for large scale internal sterilizing.

7. Cont'd

Ranger subsystems were assembled with dry box procedures utilizing ethylene oxide as gaseous sterilant and liquid antiseptics. Formaldehyde is the most promising sporicidal liquid sterilant. A satisfactory method of sterilizing electrical connectors has not yet been found. Terminal sterilization of the assembled lunar spacecraft has been achieved by 11 hr exposure of the vehicle to a mixture of 12 percent ethylene oxide, 88 percent freon-12 by weight, at room temperature and 30-40 percent relative humidity. The modified Ranger nose cone was used as a gas sterilization chamber, and as a protective shroud against recontamination before launch. The required sterility tolerance of 10^{-4} or lower for Mars landing spacecraft is extremely difficult to achieve. Terminal heat sterilization of the space vehicle in a protective shroud seems the best approach for achieving the required sterility tolerances. Many problems exist, however, in implementing this procedure.

8. INTERNATIONAL COOPERATIVE PROGRAMS, Section B,

Topic III

A Review of Space Research, Publication 1079, Chapter 15,

p. 15-14

National Academy of Sciences-National Research Council:

Washington, D. C., 1962

Additional attempts should be made to discuss with other launching countries the question of avoiding contamination of celestial bodies, especially Mars.

9. A SEISMOMETER FOR RANGER LUNAR LANDING

Lehner, F. E., Witt, E. O., Miller, W. F.,

Gurney, R. D.

Seismological Laboratory, California Institute of

Technology, Pasadena, California

NASA Contract NASw-81

May 15, 1962

Final Report, 43 pp. and 21 figs.

The unit was designed to weigh less than 10 lb and tested for an operating temperature range of -10 to +80 C. Sterilization heat soaks at 120 C for up to 36 hr are not detrimental. Proof tests of flight prototypes included heat sterilization for 24 hr at 125 C and gas sterilization for 24 hr in ethylene oxide. These sterilization tests were performed without the n-heptane instrument fluid. Stycast 10-90 or Stycast 30-50 were used as the encapsulating material for the components. On completion of the heat soak, the pulser and batteries were potted into the seismometer body with sporicidal polyester and assembly completed in a "clean room." After all performance tests were completed, the seismometers were delivered to JPL for gas sterilization and storage until required. Instruments were transferred and stored in sealed containers to prevent contamination.

10. SCIENTIFIC INSTRUMENTS IN SPACE EXPLORATION

Heacock, R. L.

Science, v. 142, pp. 188-195, 1963

Complete sterilization of the Martian space vehicle is generally agreed upon as a necessity. In contrast to the Moon or Venus, Mars seems likely to possess an atmosphere hospitable to microbial life. Therefore, no earthly organisms should contaminate the planet until biologists have had an opportunity to isolate whatever native life forms may exist there. The sterilization techniques felt necessary to insure no contamination involve heating the space vehicle to 135 C and holding this temperature for 24 hours. Surface sterilization of individual parts may be accomplished with ethylene oxide. Since this temperature is deleterious to many component materials, further research is urgently needed.

11. STERILIZING UNMANNED SPACECRAFT

Jaffe, L. D.

Astronautics and Aerospace Engineering, v. 1, no. 7

pp. 22-29, 1963

An engineering examination of the problems of sterilization in unmanned exploration is presented from the viewpoint of contamination of another planet with Earth organisms. The chance of contaminating Mars in the course of unmanned exploration should be kept low compared to the chance of contaminating it during the first manned landing. The probability of microbes being released by spacecraft during a manned landing is considered by engineers to be of the order 10^{-1} or higher. An adequately low number for the permissible probability of contamination during the unmanned program then becomes 10^{-2}. On considering the probability of failure in a program of 14 flights to Mars, a permissible probability of contamination of 10^{-4} as suggested by Hobby and the 1962 Space Science Board Study is reasonable. Since Venus represents a less favorable environment for growth of Earth organisms, the suggested assurance against releasing viable organisms into the Venus upper atmosphere is 10^{-1}. To avoid interference with experiments which may be aimed

11. Cont'd

at determining the origin of organic substances found on the Moon, the weight of viable organisms per flight in a 40-flight unmanned program should be limited to 10^{-2} g. The number of dead organisms introduced should be low. Ablation in a planetary atmosphere will not sterilize very small fragments, nor will the centers of certain materials reach sterilization temperatures. Impact, the space environment - solar ultraviolet and soft X-rays, cosmic rays, and temperatures encountered in space will not decrease the contamination probability. Limitations of chemical and heating techniques for sterilization prior to launch, during launch, and in flight are discussed.

Since dry heat for 24 hr at 135 C is considered to lower the original number of resistant organisms by a factor of about 10^{-13}, an assumption that 10^{9} organisms are present on a "clean" spacecraft leads to the probability of 10^{-4} that a viable organism remains after this heat treatment. Problems likely to be encountered during fluid filtration and aseptic assembly are noted.

54 references

12. CULTURE OF ANAEROBIC FECAL FLORA IN MEN UNDER SIMULATED SPACE CONDITIONS

Gall, L. S., Helvey, W. M.

Bacteriological Proceedings, p. 44, 1963

Anaerobic bacteria in human feces are difficult to isolate and study, so little is known about these bacteria and the factors influencing them. The research program for the man-in-space requires such information, since conditions of space travel may well influence the fecal flora and thus the health or well-being of the astronaut. Fecal samples were taken at beginning of test and twice a week for the next two weeks on four groups of six young men who were held in a chamber under varying atmospheric conditions simulating space flight and fed mainly dehydrated food. An anaerobic culturing technique similar to that used for isolating rumen anaerobes was used to study the predominating fecal flora of these subjects. Serial anaerobic culturing of fecal material was carried out to the 10^{-13} dilution, and aerobic counting plates were made of 10^{-5} to 10^{-7} dilutions. These studies showed that viable obligately anaerobic bacteria were present in 100 billion to 10 trillion per gram of fecal material in 20 of 23 subjects at all five time periods. Aerobic plate counts ranged from 100,000 to 100 million per gram of feces.

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Thus, strict anaerobes were 100 to 10,000 times more prevalent than aerobes in the feces of these 20 men. Two of the other three subjects had facultative anaerobic cocci and one man on oral antibiotics had coliforms as the predominating flora throughout the study. Preliminary studies suggest that the fecal flora of these subjects was relatively stable during the trial and that many of the bacteria isolated are different from previously described species.

13. RELAXING OF STERILIZATION REQUIREMENTS (Hearing before the Committee on Aeronautical and Space Sciences, United States Senate, Eighty-Eighth Congress, First Session, on S. 1245)

April 24, 25, 26, 29, and 30, 1963

Part I Scientific and Technical Programs, Washington, D. C., 1963, pp. 321-323, U. S. Government Printing Office, 1963

(A bill to authorize appropriations to the National Aeronautics and Space Administration for research, development, and operation; construction of facilities; and for other purposes)

Dr. Homer E. Newell, Director of Office of Space Sciences, NASA, stated to Senator Clifford P. Case, that NASA was relaxing sterilization requirements for lunar vehicles. This was being done because the present state of sterilization techniques called for heating the vehicle to a high temperature over a long period of time. This has the effect of aging the vehicle prematurely and thus lowers its reliability. In the opinion of many biologist, lower sterilization requirements will not harm biological experimentation of the Moon since the greatest opportunity lies at a great depth below the lunar surface. There can be no

13. Cont'd

spread of infection from an unsterile vehicle since there is no atmosphere present on the Moon and the lunar surface is exposed to high vacuum, irradiation by ultraviolet light and X-rays, lethal particle radiations, and very high temperatures. It was noted that relaxation of sterilization requirements must not happen in the case of the Mars probes lest the possibility of determining the presence of life be destroyed.

14. COMMENTS AND QUESTIONS SUBMITTED BY SENATOR MARGARET CHASE SMITH ON STERILIZATION OF SPACE VEHICLES FOR LUNAR LANDINGS (Hearings before the Committee on Aeronautical and Space Science, United States Senate, Eighty-Eighth Congress, First Session on S. 1245)
April 24, 25, 26, 29, and 30, 1963
Part I Scientific and Technical Programs, Washington, D. C., 1963, pp. 596-600, U. S. Government Printing Office,
(A bill to authorize appropriations to the National Aeronautics and Space Administration for research, development, and operation; construction of facilities; and for other purposes)

Senator Smith was concerned about NASA decisions to relax arrangements for the sterilization of space vehicles for lunar landings. It was felt that these decisions violated agreements made by the United States with the Committee on Contamination of Extra-Terrestrial Exploration of the International Council of Scientific Unions. It was pointed out by Dr. Robert C. Seamans, Associate Administrator, NASA, that CETEX, at the second meeting in 1959 had taken the position that the presence of any life on the Moon was

14. Cont'd

extremely unlikely and that "it may not be possible to avoid all types of contamination." As far as dead contaminants of extraterrestrial nature were concerned, danger to future studies could be reduced by limiting areas for landing on the Moon. No plans are being made for sterilizing manned vehicles to the Moon. There does not seem to be any great danger of contaminating the Earth with lunar organisms because it is extremely unlikely that organisms exist on the Moon which would thrive in Earth environment. No relaxation of sterilization requirements is forseen for planetary landings. By 1966, sterile and technologically reliable spacecraft are planned for the Mars opportunity. The current state of sterilization technology is now considered inadequate, but it is expected to be sufficiently advanced by late 1966.

15. THERMAL CONTROL IN SPACE VEHICLES

Alexander, A. L.

Science, v. 143, pp. 654-660, 1964

Temperatures of satellites and spacecraft must be controlled reliably to satisfy requirements of internal instruments and payloads. Restrictive temperature limitations of electronic components dictate the thermal design of the vehicle. Transistor networks are useful only between 0 and about 60 C. Some batteries operate efficiently only within narrow temperature limits. Nearly all biological processes must be maintained within a range about 40 C. The highest temperature allowed in manned spacecraft is 43 C.

16. ABL MARS LANDING MAY COME IN 1971

Missiles and Rockets, v. 15, no. 8, p. 15, 1964

The director of NASA's office of Bio-sciences indicates it will probably be 1971 or 1973 before the U. S. lands a large integrated Automated Biological Laboratory (ABL) on Mars. Other NASA sources consider a 1971 launch of a large ABL with the Saturn V booster as improbable due to high cost and problems with sterilization methods which have to be solved. Any ABL mission will be part of the Advanced Voyager program.

17. VIEWS ON SPACE PROBE STERILIZATION

Hedén, C.-G.

Part I, pp. 1-14

Background paper, COSPAR Consultative Group on Potential Hazards of Space Experiments,

Meeting, Geneva, February 14, 1964

The problem of sterilizing and decontaminating rockets which may impact a planet or the lunar surface is of concern to the world's biologists. Therefore, since 1958 CETEX and COSPAR have been working on a code of procedures to be followed by launching stations. Exact quantitative specifications have been difficult to establish since no positive data exists on the rate of growth of organisms under conditions believed to exist on Mars or Venus. Also no clear understanding exists of the sterilizing effect of space conditions and reentry into the atmosphere. Experimental programs to acquire more data on these points have been recommended. Attention has been given to collecting and identifying organisms in space and the difficulty of deciding whether their source is extraterrestrial. Since political considerations might prevent the necessary dialogues between Russian and American engineers and microbiologists, the

17. Cont'd

construction of a testing device or "mock cone" consisting of heat sterilizable and non-heat sterilizable components is also recommended.

18. SPACE QUARANTINE COMMENTS MADE IN OR ENCLOSED WITH CORRESPONDENCE

Hedén, C.-G.

Views on Space Probe Sterilization, Part I, Appendix I, Background paper, COSPAR Consultative Group on Potential Hazards of Space Experiments,

Meeting, Geneva, February 14, 1964

The Space Science Board of the National Academy of Sciences, issued a statement of policy in 1963 which stated 1) "that an immediate study program be undertaken to determine sterilization requirements for space probes and to develop recommendations, compatible with present design and assembly processes regarding necessary sterilization procedures" and 2) "that procedures be immediately established and implemented to insure a complete inventory of all components of all space probes." NASA formulated the policy of "sterilizing, to the extent technically feasible, all space probes intended to pass in the near vicinity of or impact on the Moon or planets." The Space Science Board has adopted a two-part policy concerning 1) lunar probes, 2) Mars probes. Although the lunar surface is an extremely unfavorable environment for the growth of terrestrial organisms, the Board recommends that contamination be kept minimal because

18. Cont'd

of the lack of knowledge concerning lunar subsurface conditions. The cleanliness level of the probe should approximate that which prevails in most hospital surgical rooms. Mars, however, represents a possibly more hospitable environment, and the sterilization procedures should be more stringent. All scientists whose correspondence is quoted stress the "catastrophe" to biological studies if a Mars landing is made without sterilization of the space vehicle and a means for incorporating a life-detection experiment in the initial landing. Procedures of sterile construction and decontamination before launch are given. An international committee of scientists should be established to approve the sterility of space vehicles.

19. WORKING DOCUMENT FOR THE CONSULTATIVE GROUP

Hedén, C.-G.

Views on Space Probe Sterilization, Part I, Appendix 4, Background paper, COSPAR Consultative Group on Potential Hazards of Space Experiments,

Meeting, Geneva, February 14, 1964

The Consultative Group on Possible Hazards of Space Experiments recommends that COSPAR request the United Nations to declare Mars a Temporary Biological Preserve to be approached only by spacecraft subjected to certified sterilization procedures. Such procedures must be proven effective both for surface sterilization and for destroying or removing viable, resistant spores entrapped between solids or contained in liquids which are not themselves sterilants. Less than 10^{-4} probability that a single living organism is released on the planet's surface should be the goal. These specifications should be the concern of a Control Sterility Group established as a unit within the organisation responsible for planetary missions.

20. CONTROLLED CONTAMINATION OF HERMETICALLY SEALED ELECTRONIC COMPONENTS OF SPACE VEHICLES FOR DEVELOPMENT OF TERMINAL STERILIZATION PROCEDURES

Miller, A. K.

Lockheed Spacecraft Sterilization Systems,

Van Nuys Facility

Lockheed Missiles and Space Company

Sunnyvale, California

Final Report

Contract AF41 (609)-1544, Project No. 7930, 34 pp.,

December 31, 1962

Controlled contamination of components during manufacture was accomplished to support studies to determine whether normal manufacturing techniques were sufficient to sterilize, and what sterilization procedures could be used without affecting component characteristics. Approximately 375 components were inoculated with Bacillus stearothermophilus spores and 230 components were inoculated with B. subtilis var. niger spores. Heat sensitive and heat resistant resistors, capacitors, potentiometers and diodes of various internal composition were selected. Details of component manufacturing processes are given, and a recommendation is made that manufacturing methods should include aseptic assembly

20. Cont'd

coordinated with in-line sterilization to achieve internal sterilization of components.

21. STERILIZATION OF LUNAR AND PLANETARY SPACE VEHICLES - A REVIEW

Clemedson, C.-J.

In "International Astronautical Congress, 13th Varna, Bulgaria , September, 1962, Proceedings, Volume I", Edited by N. Boneff and I. Hersey , pp. 292-313, Springer-Verlag, Vienna, 1964

Dr. Clemedson has not replied to a letter sent in 1963 concerning this paper. The publication is not available for abstracting. A citation referring to a preprint of this article noted 87 references.

22. PROCEDURES NECESSARY FOR THE PREVENTION OF PLANETARY CONTAMINATION (Presented at the Fifth International Space Science Symposium, COSPAR, May 12 - 16, 1964)

Hall, L. B., Bruch, C.

Florence, Italy

Studies of microbial survival in simulated deep space conditions have established that these conditions will not sterilize contaminated spacecraft. Likewise, data presented at this and previous meetings of COSPAR have shown that many terrestrial microbes, particularly anaerobic sporeformers, readily tolerate simulated Martian environments. Any spacecraft landing on Mars must have a low probability of harboring any terrestrial organisms, especially sporeformers, if the goals of exobiology are not to be compromised.

Before the planning, production and monitoring of sterile spacecraft can be undertaken, the theory and principles of sterilization and their application to the special problem of spacecraft sterilization must be understood. The response of a given population of microorganisms to sterilizing agents is affected by several factors; these factors in turn regulate the kinetics of the killing process. The only processes that achieve both surface and interior sterilization are heat and

22. Cont'd

radiation. Of these, dry heat is the agent of choice. If all parts of the spacecraft are assembled under the most rigorous conditions of cleanliness, the capsule can be brought to terminal sterilization containing a total of not more than 10^5 viable organisms. These can be killed by exposure to 135 C for 24 hours with a confidence of greater than 10^4.

Spacecraft must be specifically designed to withstand such heat sterilization without reduction of reliability. Labile parts must be kept to a minimal number, sterilized by methods other than heat, and inserted into the sterilized spacecraft by sterile techniques. The entire process must be under very tight control with tests and records of every detail. The sterilized spacecraft must be encapsulated and remain therein during final testing and launch until the capsule is opened in deep space. The design and production engineer must understand how all these procedures will affect the spacecraft, and the biologist must constantly monitor and educate the engineers on the scope and limitations of these various sterilizing procedures.

Authors Abstract

23. THE OLIGODYNAMIC ACTION OF METALS

Lutaeva, A. I.

Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (USSR), v. 19, pp. 165-175, 1937 (In Russian)

Review with 64 references.

24. EFFECT OF β-PROPIOLACTONE ON METABOLISM OF PSEUDOMONAS AERUGINOSA AND GROWTH OF CERTAIN FUNGI

Bernheim, F., Gale, G. R.

Proceedings of the Society for Experimental Biology and Medicine, v. 80, no. 1, pp. 162-164, 1952

β-propiolactone (BPL) at 0.25 mg/ml inhibits the oxidative metabolism of a strain of Pseudomonas aeruginosa. In aqueous solution, the lactone hydrolyzes to β-hydroxypropionic acid. The lactone may be used as a surface disinfectant since it is fungistatic as well as bacteriostatic, but it would be necessary to store and apply it in a nonaqueous medium. Slow hydrolysis would keep the surface acid, and the combined effect of the lactone and acidity should inhibit growth. Because of low toxicity in dilution, BPL could be applied frequently without danger of systemic effects from absorption through the skin.

25. DECONTAMINATION OF ENCLOSED SPACES WITH BETA-PROPIOLACTONE VAPOR

Bruch, C. W.

The American Journal of Hygiene, v. 73, no. 1, pp. 1-9, 1961

Beta-propiolactone (BPL) was aerosolized into several rooms and buildings which had a variety of surface finishes, equipment, room volumes, and history with respect to bacterial contamination. The lactone was used on the basis of 10 mg per 1 of space for a contact time of 2-4 hr followed by 6-18 hr charcoal adsorption or ventilation. Spore strips of Bacillus subtilis var. niger and B. stearothermophilus as well as swab samples were used to measure the effectiveness of the decontamination procedure. The relative humidity was raised when necessary since BPL is not effective as a sterilant at less than 70 percent relative humidity. Disinfection was usually complete when measured by the sterilization of spores on paper strips, but one treated space had residual counts of 5-25 spores from the initial 100,000 B. subtilis var. niger spores. Liquid and vapor BPL will corrode or cause deterioration of certain metals and plastics.

26. CHEMICAL AND COMBINED METHODS FOR PLASMA STERILIZATION

LoGrippo, G. A., Hartmann, F. W.

Bibliotheca Haematologica, v. 7, pp. 225-230, 1958

Over 600 chemicals were tested for virucidal activity. Only five compounds were suitable for inactivating viruses in human plasma. These were paraformaldehyde, ethylene oxide, butylene oxide, sulfur mustard and beta-propiolactone (BPL). BPL was considered the most promising drug for chemical sterilization of plasma. BPL hydrolyzes rapidly in water and plasma to sodium beta-chloropropionate and sodium hydracrylate, both relatively nontoxic compounds. In order to ensure maintenance of plasma protein integrity at minimal BPL levels, combinations of BPL with other virucidal agents were studied to find additive or synergistic virucidal action without additional protein alteration. BPL and UV irradiation was found to be most satisfactory. A BPL concentration of 500 mg/l combined with 2 milliwatts/sq cm UV irradiation eliminated virus activity while BPL alone required 3500 mg/l and UV alone required greater than 8 milliwatts/sq cm. The virucidal concentration of the combination is seven times lower than that of BPL alone and three to four times lower than that of UV alone.

27. A GENERAL REVIEW OF CHEMICAL STERILIZATION IN SPACE RESEARCH

Opfell, J. B.

In "Life Sciences and Space Research", v. II, A Session of the Fourth International Space Science Symposium, Warsaw, June 3-12, 1963, pp. 385-405, North-Holland Publishing Company, Amsterdam, 1964

Chemical sterilization by itself is not a sufficient technique to produce sterile assemblies of the complexity found in spacecraft. It provides alternatives and supplementation to contamination control, cleaning, irradiation, and exposure to elevated temperatures. Because of limitations in establishing sterility with chemical agents, physical agents such as heat or radiation will be given first consideration in the design of sterile spacecraft components. Chemical sterilization should be considered only if these agents are not compatible with component materials or the performance of the components.

Gaseous sterilization is an application of chemical sterilization in which the sterilizing agent is in the gas phase when in immediate contact with the organism to be destroyed. In general, liquid sterilants act about ten times more rapidly than do gases. In quasi-gaseous processes, the chemical agent is dispersed as a vapor or aerosol which condenses on all accessible surfaces.

27. Cont'd

Penetration of the agent into confined spaces is limited. Both beta-propiolactone and formaldehyde behave as gas sterilants at temperatures which prevent condensation. These temperatures may be intermediate between those at which ethylene oxide is normally used and those of conventional steam sterilization processes. In combination with low pressure steam, surfaces may be sterilized at temperatures below those used for steam sterilization.

One of the most useful applications of chemical sterilization will be the preparation of sterile environments for sterile assembly, adjustment and calibration of components and even the space probe itself. The enclosure and packaging materials can be designed for compatability with the gas sterilant or quasi-gaseous sterilant. Immersion of objects in liquid sterilants may be useful in sterile assembly activities.

Objects which cannot be exposed to sterilizing doses of radiation or to sterilizing temperatures must be supplanted with materials which can be treated, or they must be prepared from self-sterilizing formulations. Chemical sterilants will be used in controlling microbes and infections in manned spacecraft, and in decontaminating specimen containers returning from space exploration.

27. Cont'd

Three important design considerations are: 1) materials of construction must be compatible with the chemical agents; 2) ensure the absence of hermetically sealed surfaces harboring live microbes; 3) preserve sterility by suitable assembly techniques or packaging. Viable cells can be trapped in the mating of close-fitting parts, in adhesives, potting compounds, paints, lubricants, and solid propellants.

The state of sterility is not subject to measurement in other than a statistical sense. Certified sterility is a useful engineering concept in which a set of procedures can give an acceptable level of assurance that the residual viable microbial population on and in an object does not exceed a certain size. Confidence in process effectiveness cannot be extended to large populations of microorganisms of species whose resistance to destruction by the process have not been determined. Little common agreement exists on what methods should be used to test and evaluate sterilants. Certified sterility will not propose that the spacecraft is free of living matter or does not have a large population of microorganisms. The undetected failure of one of the set of procedures could permit failure of the entire sterility protection system.

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The vehicle for a liquid sterilant can influence the sterilization process because of partition phenomena involving the chemical agent, the cell, and the phase surrounding the cell. A liquid sterilant must not evaporate during the time required to sterilize the surface in contact. At room temperature and slightly above, water vapor, ethylene oxide, or formaldehyde are not individually effective sterilants for many microorganisms. Appropriate ratios of water vapor and ethylene oxide or formaldehyde sterilize effectively. Sterilization procedures must be effective under the conditions prevailing during manufacturing and assembly.

Fresh formaldehyde monomer in absolute methanol appears to be an effective sterilant generally compatible with materials, particularly electrical devices. Formaldehyde, beta-propiolactone, and ethylene imine may change the properties of fibrous materials. Liberation of absorbed formaldehyde long after completion of sterilization could result in deposition of paraformaldehyde on electrical or optical equipment and interfere with performance.

Laboratory demonstration of sterilant compatibility with materials to be sterilized is almost essential in view of the variety of factors which influence the sterilant-material interaction. Use of a gas sterilant under pressure may shorten the exposure time by increasing the effective concentration. For each temperature, however, there is a concentration above which no

27. Cont'd

shortening of time is possible. Raising the temperature enhances the effectiveness of the alkylating agents but lowers the effectiveness of certain oxidizing agents. Reduction in absorption of the sterilant by the cell may occur.

A sequence of chemical treatments can improve the efficiency of the sterilization process. Should terminal surface sterilization require ethylene oxide, gloveboxes for sterile assembly can be sterilized with ozone, washed with liquid sterilant, or exposed to a beta-propiolactone fog.

A chemical sterilant does not always act linearly with time. The F and D values which describe the rate of viability loss in microbial populations compare the kinetics of processes rather than their endpoints. Extrapolation to processes for destroying very large microbial populations is not recommended.

Bacterial spores are less resistant to alkylating agents than to other chemical sterilants. Biological alkylating agents include formaldehyde, sulfur and nitrogen mustards, methanol amides, methane sulfonyloxy derivatives, certain lactones, the epoxides, and ethylene imines and sulfides. The alkylating agents have been termed 'radiomimetic' since many of their biological effects correlate with those of radiations. The distinct advantages of ethylene oxide over other alkylating agents at low temperatures are the ability to penetrate porous materials and the lack of a

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requirement for excessive humidity for sterilization. Para-formaldehyde, a beta-propiolactone polymer, and ethylene imine have imparted self-sterilizing properties to plastics and potting compounds.

Engineering developments will establish the activity of different amounts of sterilant and vehicle on materials and sterilization effectiveness. Methods of mixing the chemicals in materials to be self-sterilizing, the diffusion rates of chemical agents into and out of materials and spaces, the solubility and corrosiveness will be determined. Organisms which present most difficulties in sterilization are usually associated with dust or soil, both of which influence the environmental and process requirements to produce sterility.

76 references

28. DEMONSTRATION OF INTERACTION BETWEEN PAIRS OF ANTIBACTERIAL AGENTS

Hugo, W. B., Foster, J. H. S.

Journal of Pharmacy and Pharmacology, v. 15, p. 79, 1962

Pairs of compounds used as bacteriostatic agents were tested against six bacterial species to determine whether the combinations were more or less effective than either one alone or if no interaction occurred. The technique used paper strips loaded with the compounds. These were placed at right angles on a seeded agar plate. The zones of inhibition and growth pattern between the strips indicated the mutual effect of the combination tested. The interaction of 28 combinations of phenylmercuric nitrate, 2-phenylethanol, chlorcresol, thiomersalate, chlorhexidine, benzalkonium chloride, chlorbutol and eye-drop solution B.P.C. were tested against Pseudomonas aeruginosa, NCTC 7244, Streptococcus pyogenes, NCTC 8708, Staphylococcus aureus, NCTC 4163, Escherichia coli, NCTC 86, Bacillus subtilis, NCTC 8236, and Proteus vulgaris, NCTC 4636. No antagonism was demonstrated between any pair of bacteriostats. There was evidence of mutual enhancement of activity between 2-phenylethanol and the organic mercurials.

29. DECONTAMINATION OF CHICKEN EGGS USING PERACETIC ACID

Smith, C. K.

Bacteriological Proceedings, p. 147, 1964

Previous production of axenic chickens has involved the sterilization of the shell of the eggs while they were passed through a bath containing $HgCl_2$ (1:100). Peracetic acid has been used to sterilize inert surfaces of isolators and related equipment. The use of peracetic acid to sterilize the shell surfaces of eggs is an extention of these techniques and eliminates the use of a germicidal trap as well as the less adequate $HgCl_2$. Eggs containing 17-, 18-, and 19-day-old chick embryos were passed in solutions of anionic detergent and peracetic acid. These eggs were then passed into a sterile isolator through a standard vapor lock door and a spray of 2 percent acid. After this treatment, clutches of eggs produced hatches of axenic chicks. Exposure of the embryonated eggs to peracetic acid for longer than 25 min or excessive fumigation of the lock tended to reduce the hatchability. Partial neutralization of the peracetic acid to pH 5.0 increased the hatchability 71 percent when using a standard 30-min exposure period. Experiments in which the concentration of the peracetic acid was reduced to 1 percent were unsuccessful in producing axenic chicks. The organisms isolated from contaminated

29. Cont'd

clutches were *Staphylococcus epidermidis*, *Escherichia coli* *Streptococcus faecalis*, and *Alcaligenes bookeri*.

30. MICROBIOLOGICAL ASPECTS OF THE PARTIAL STERILISATION OF SOIL BY CHEMICALS. A REVIEW OF THE LITERATURE

Hall, N. M., Clegg, L. F. L.

Proceedings of the Society of Applied Bacteriology, v. 2, pp. 105-118, 1949

Bacteria in soil treated with a chemical sterilant are reduced in number for several days and then increase, usually within a few weeks, to numbers exceeding those in untreated soil. The bacteria then decrease toward the original numbers. The decrease may be very slow, taking over a year. Toluene, formaldehyde, and chloropicrin markedly repress soil fungi. Actinomycetes, on the other hand, may be stimulated by toluene and chloropicrin. Sodium cyanide (200 ppm) prevents actinomycete growth. Formaldehyde is, apart from steam, considered the best known sterilant for general purposes, but does not completely control certain plant pathogens.

74 references

31. EVALUATION OF A NEW AERIAL GERMICIDE AGAINST AIRBORNE STAPHYLOCOCCUS AUREUS

Andersen, A. A.

Bacteriological Proceedings, p. 162, 1963

A new aerial germicide consisting of an active ingredient, 2-chloro-4-phenylphenol, incorporated in a pressurized container with a solvent and a propellant, is evaluated against aerosols of Staphylococcus aureus and spores of Bacillus subtilis. The method of evaluation consisted of determining the decay rate of the bacterial aerosols in a small chamber with and without the aerial germicide being present. The method is unique in that the effect of very short exposures of airborne bacteria to aerial germicides can be measured more precisely. By use of the method outlined extremely potent aerial germicides may be evaluated against highly sensitive airborne microorganisms. The procedure requires a minimum of time. The equipment is simple and relatively inexpensive. The 2-chloro-4-phenylphenol aerosol was found to be extremely effective against airborne Staphylococcus aureus. 2.2 micrograms of the active substance per liter of air completely eliminated aerosols of 52,000 staph particles per liter in two minutes. The germicide was only moderately effective against spores of Bacillus subtilis. In areas of

31. Cont'd

the hospital that could be closed for short periods such as operating rooms, clothes chutes, etc., where it is extremely important to reduce the staph count to a minimum or where it is known that high counts of staph are found it should be possible to eliminate or greatly reduce the staphylococci with this aerial germicide.

32. GERMICIDAL EFFECTS OF ALCOHOLS

Coulthard, C. E., Sykes, G.

Pharmaceutical Journal, v. 137, pp. 79-81, 1936

(Abstracted in Chemical Abstracts, v. 31, p. 5014[7])

Sporicidal action of 70 percent alcohol is increased by addition of 1 percent sodium hydroxide, potassium hydroxide, hydrochloric acid, sulfuric acid, phosphoric acid, or of 10 percent ammonia - m-cresol.

33. DESTRUCTION OF BACILLUS SUBTILIS SPORES WITH SOLUTIONS OF SODIUM HYDROXIDE

Whitehouse, R. L., Clegg, L. F. L.

Journal of Dairy Research, v. 30, pp. 315-322, 1963

The destruction rate was studied over the temperature range 34-82 F to obtain information applicable to immersion cleaning of dairy utensils and to consider the nature of the disinfection curves upon slowing the rate of disinfection. A three dimensional graphical analysis showed that an increase in temperature from 34 to 82 F had a more significant effect on spore destruction than an increase in sodium hydroxide concentration from 1.5 to 5.0 percent. The overall effect could be expressed as $t = 2.6 \times 10^8 / C^{1.66} \theta^{3.79}$, where t = time in hours for 99 percent destruction of spores, C = sodium hydroxide concentration expressed as percent weight/volume, and θ = temperature in Fahrenheit. Since t for a 2 percent sodium hydroxide solution at 34 F is 5 times that of a 5 percent solution, immersion cleaning in cold climates might be assisted by increasing the sodium hydroxide concentration from the normal 2-3 to 5 percent. Disinfection tests at low temperatures were rather variable in results and prevented a careful study of the nature of disinfection curves although such curves are essentially sigmoidal.

34. THE CHEMICAL ENHANCEMENT OF BACTERIAL RADIO-SENSITIVITY IN THE RADIATION STERILIZATION OF FOODS

Phillips, A. W.

September 1, 1955 - December 31, 1957

Contract No. DA 19-129-QM-524

S-546 Report No. 12, 67 pp.

Final Report

Several compounds were found to be synergistic or complementary with X-irradiation in inactivating spores of Bacillus thermoacidurans, B. subtilis, and B. cereus. Ethylene oxide and propylene oxide had synergistic activity with B. thermoacidurans and B. subtilis. Alpha-amino-n-butyric acid was synergistic with radiation toward B. cereus spores. Compounds found to be complementary with X-irradiation in spore inactivation include sorbic acid, sodium diacetate, 1,2-propanediol, benzalkonium chloride, malonic acid, maleic acid and sodium desoxycholate. B. subtilis spores were more radioresistant than those of B. cereus or B. thermoacidurans. The respective D_o values were about 150,000, 80,000 and 50,000 rad. Milk and fresh meats were sterilized by combining radiation and ethylene oxide treatment using 100,000 rad or less radiation. An effective synergist should probably reduce the sterilizing dose from 2×10^6 rep to 10^5 rep or less.

35. DESTRUCTION OF BACTERIA

Wehrle, T.

Swiss Patent No. 214,287, July 1, 1941

(Abstracted in Chemical Abstracts, v. 36, p. 4942^5)

Trioxyethylene is heated in vacuo to produce formaldehyde vapor. Steam is introduced to hinder polymerization of formaldehyde and to lessen the distillation of trioxyethylene. The vapor mixture of formaldehyde and steam is injected into a vacuum chamber in which disinfection takes place.

36. THE BACTERICIDAL ACTION OF OZONE SOLUTIONS

Wuhrmann, K., Meyrath, J.

Schweizerische Zeitschift fuer Allgemeine Pathologie und Bakteriologie, v. 18, pp. 1060-1069, 1955

Carbonate and bicarbonate buffers were saturated with ozone-air mixtures. The contact time required to kill 99.99 percent of Escherichia coli or spores of the Bacillus megaterium-cereus group was determined. At 12 C and pH 7.0, 20 gamma/l of silver, chlorine, or ozone killed 99 percent of E. coli in 180, 1.5, and 0.2 min respectively. Ozone killed the spores 300 times faster than similar concentrations of chlorine dioxide or hypochlorite. A plot of the log of the time required to kill 99.99 percent of the bacteria vs the log of the ozone concentration was a straight line. Spores which were stored at room temperature for over 40 days had a greater resistance to ozone.

37. STERILIZATION OF DRY GELATIN

Mayr, G., Kaemmerer, H.

Mitteilungen der Versuchsstation fuer das Gaerungsgewerbe sowie des Institutes fuer Angewandte Mikrobiologie, v. 11, p. 9, 1957 (In German)

(Abstracted in Chemical Abstracts, v. 51, p. 13249 g, 1957)

Dry gelatin can be sterilized with gaseous mixtures of ethylene oxide and propylene oxide or ethylene oxide and methylene bromide in concentrations of 1000 g per cubic meter at temperatures from 25 to 30 C and a relative humidity of 55 to 70 percent.

38. STERILIZATION OF DRY EGG POWDER WITH ETHYLENE OXIDE

Mayr, G., Kaemmerer, H.

Mitteilungen der Versuchsstation fuer das Gaerungsgewerbe sowie des Institutes fuer Angewandte Mikrobiologie, v. 11, pp. 9-11, 1957 (In German)

(Abstracted in Chemical Abstracts, v. 51, p. 13249 g, 1957)

Powdered dry eggs can be satisfactorly sterilized with ethylene oxide in a concentration of 750 g per cubic meter within 6 hours. No influence on the proteins and fatty acids was observed.

39. A COMPARATIVE PROCEDURE FOR EVALUATING ANTI-MICROBIAL ACTIVITY OF GASEOUS AGENTS

Pan, C.-H., Gast, J. H.

Bacteriological Proceedings, p. 53, 1959

Two pieces of apparatus have been designed for testing anti-microbial activity of gases, one permitting direct gaseous exposure of organisms on a millipore filter, the other allowing incubation with shaking and measurement of absorbance of the suspension. The procedure with the different gases and microorganisms tried is as follows: The bacterial suspension, adjusted to an optical density of 0.1, is filtered at uniform negative pressure and both filter pad and filter are transferred to a petri dish exposure vessel. The gaseous agent, introduced in a metered stream of carrier gas, is tested at different concentrations and exposure times. The filter containing the exposed organisms is transferred to the growth chamber from which O.D. measurements are made by inverting the suspension into the attached absorbance tube. The same volume of fluid as used in the original suspension is added, the vessel shaken for 30 sec, and the absorbance measured. Optical density readings identical with the starting values are obtained by proper handling. The growth chamber is incubated and shaken for two hr in a Dubnoff shaker and optical density readings made at intervals. Using 1.0 mg/liter dry

39. Cont'd

formaldehyde in either carbon dioxide or air, and 5 percent ethylene oxide in carbon dioxide no growth was observed after two hr from filters containing Escherichia coli exposed for 15 min. Controls exposed similarly to carrier gas alone reached an optical density of 0.3. Intermediate values were obtained with shorter exposure times or lower concentrations of gas.

40. REACTION OF THE STERILANT, ETHYLENE OXIDE, ON PLASTICS

Tessler, J.

Applied Microbiology, v. 9, no. 3, p. 256, 1961

Ethylene oxide is used to sterilize surfaces and objects that cannot be treated with heat or corrosive chemicals. Plastic parts of equipment considered contaminated with a virus were badly damaged and softened following 5-hr exposure to an ethylene oxide (11 percent) - Freon 11 (44.5 percent) - Freon 12 (44.5 percent) gaseous sterilant after prehumidification. Samples of the thermoplastics were obtained and subjected to the gaseous sterilization procedure. Plastics were also placed in Freon 11 for 5 hr at 37 C and in liquid ethylene oxide at 25 C for 4 hr. The following table summarizes the results.

	Damaged by		
Plastics	Gas Sterilization	Freon 11	Liquid Ethylene oxide
Tenite (Cellulose acetate butyrate)	Yes	No	Yes
Styron 480 (polystyrene resin)	Yes	Yes	Yes
Zytel 101 (nylon resin)	No	No	No

40. Cont'd

The stability of plastics contemplated for exposure to a gaseous sterilant should be determined under conditions which will indicate gross damage to the material.

41. THE RELATIONSHIP BETWEEN THE AGE OF BACILLUS SUBTILIS SPORES AND THEIR RESISTANCE TO ETHYLENE OXIDE

Bomar, M.

Folia Microbiologia, v. 7, pp. 259-261, 1962

Sporulated cells of Bacillus subtilis strain 71 were dried on 3 mm glass beads and incubated for 2-14 days at 0 and 92 percent relative humidity at 20 C. The beads (1.6-2.6 X 10^7 cells per bead) were then exposed to ethylene oxide in 300 ml blood bottles by flowing ethylene oxide through the bottles at a rate of 0.1-0.2 cu m per min and then sealing the bottles. Spore survival was studied qualitatively by placing beads in glucose nutrient broth and examining the tubes for turbidity during 6 days incubation at 30 C. Quantitative tests were performed by placing beads in molten agar and after agitating beads in distilled water. Spores were exposed to ethylene oxide for 6 hr at 20 C prior to culturing the beads. It was found that spores stored for 8-14 days had far greater resistance to ethylene oxide than 0-4 day-old spores. Results with spores stored at both conditions of humidity were similar. Spores stored at 92 percent relative humidity for 4 days prior to ethylene oxide exposure had zero survivors while spores maintained for 8 days prior to exposure had 4.7 X 10^3 survivors. The controls in each preparation had 81 and 90 X 10^6 spores respectively.

42. PENETRATION BY GASES TO STERILIZE INTERIOR SURFACES OF CONFINED SPACES

Opfell, J. B., Wang, Y.-I., Louderback, A. L.

Miller, C. E.

Applied Microbiology, v. 12, no. 1, pp. 27-31, 1964

A theoretical discussion is presented concerning the penetration of ethylene oxide through a small orifice into confined spaces filled with air at atmospheric pressure and devoid of convection currents. The diffusion coefficient, D_v, for several gases was calculated. D_v for beta-propiolactone (0.337 ft^2/hr; 80 F) is almost as large as that for ethylene oxide (0.482; 80 F). The low penetrating ability of beta-propiolactone is due to its low saturation pressure in the vapor state. This property also limits penetration of formaldehyde vapor at temperatures below the decomposition temperature of paraformaldehyde. Data is given for predicting the minimal time required for the concentration of a sterilizing gas to reach a certain level at a remote surface in a confined space. For rapid penetration, convection in addition to diffusion is essential.

13 references

43. EVALUATION OF STERILIZATION BY GASEOUS ETHYLENE OXIDE

Stierli, H., Reed, L. L., Billick, I. H.

Public Health Monograph No. 68, Public Health Service Publication No. 903, U. S. Department of Health, Education, and Welfare. 1962

A review and evaluation of ethylene oxide sterilization procedures is presented. Doubling the ethylene oxide concentration reduces the sterilization time about one-half. Bactericidal activity increases by a factor of 2.74 for each 10 C rise within the range 5-37 C. Humidity is an important factor in the killing action. B. subtilis var. globigii spores at 28 percent relative humidity are killed 4 times as rapidly as at 65 percent and almost 10 times as rapidly as at 97 percent relative humidity. A variety of bacteria, spores, and pathogenic fungi were exposed to a mixture of 11 percent ethylene oxide and 89 percent halogenated hydrocarbons in this study by utilizing a gas sterilizer in which the material was subjected to an initial vacuum, exposed to the gas sterilant under about 15 lb pressure for 2 or more hours at 132-140 F, and removed from the chamber after a final vacuum treatment to remove the gas. All organisms were killed in two hours in the presence of 1000 mg/l ethylene oxide at 30-50 percent relative humidity. Medical equipment and other objects were

43. Cont'd

contaminated and exposed to sterilizing cycles. Erratic results with contaminated hypodermic needles was attributed to protection of cells in a grossly contaminated area by cells in the surface layer which limited diffusion of the gas. Properly cleaned needles would be sterilized without difficulty.

26 references

44. ETHYLENE OXIDE STERILIZATION OF TISSUE CULTURE MEDIA

Brown, B. L., Fuerst, R.

Science, v. 142, pp. 1654-1655, 1963

Under conditions of 60-80 percent relative humidity, 37 C, and an exposure time of at least 24 hours, 1 percent by volume of ethylene oxide killed large inoculums of Aerobacter aerogenes, Escherichia coli, and Bacillus megaterium bacteriophage which had been added to the tissue culture media. A yeast-like organism isolated from a contaminated HeLa cell culture was also killed. It is proposed that ethylene oxide be considered for sterilization of heat-sensitive chemicals added to tissue culture media, particularly if such substances are used in small quantities.

45. KINETICS OF BACTERICIDAL ACTIVITY OF ETHYLENE OXIDE IN THE VAPOR PHASE. I. EFFECTS OF CELLULAR WATER ACTIVITY

El-Bisi, H. M., Vondell, R. M., Esselen, W. B.

Bacteriological Proceedings, p. 13, 1963

Comparative resistance study was conducted among selected bacteria, exposed to dry ethylene oxide vapor (ETO) at 60 C and 5 psig. Vapor death rates (VDR) of desiccant-dry populations increased with residual bound water content. S. faecalis exhibited markedly higher resistance than all sporogenous types (C. sporogenes, B. stearothermophilus, B. subtilis var. niger and B. coagulans, in a descending order). When water-suspended populations were exposed, D values were 10, 41, 32, 95, 92 and 42 sec respectively. No relation was noted between relative resistance to ETO and that to moist heat. B. subtilis and S. faecalis were selected for studying effect of preconditioned cellular water activity (a_w). Sorption-desorption rates were determined to establish appropriate parameters for preconditioning and correlate such behavior with VDR. Contrary to S. faecalis, B. subtilis sorped moisture at higher rates than for desorption. When preconditioned at increasing a_w levels then exposed to dry ETO, death was initially accelerated

45. Cont'd

through varying intervals, then VDR curves tailed out indefinitely. Preconditioning B. subtilis up to 0.87 a_w rendered population as sensitive as that exposed in a water-suspended state, whereas S. faecalis required preconditioning at 100 percent RH to acquire equivalent sensitivity. Effect of cellular water activity, therefore, is a function of desorption rate of contaminating flora, initial population level and inherent death rate. One decisive factor which remains undetermined is the contaminated material, its sorption-desorption characteristics and bulk/vapor ratio.

46. KINETICS OF BACTERICIDAL ACTIVITY OF ETHYLENE OXIDE IN THE VAPOR PHASE. II. EFFECT OF STERILANT HUMIDITY

El-Bisi, H. M., Vondell, R. M., Esselen, W. B.

Bacteriological Proceedings, p. 13, 1963

This phase of study was aimed at determining the independent effect of sterilant humidity and its interaction with cellular water activity. Vapor death rates (VDR) were established for desiccant-dry populations exposed to ethylene oxide vapor (ETO) at: 5 psig, 60 C and different relative humidities (percent RH). All VDR curves conformed with the kinetics of 1st order reaction. For B. subtilis at dry, 35, 45, and 55 percent RH; D values (2.3 k^{-1}) were 312, 54, 38, and 57 sec respectively. Optimum VDR was attained at 45 percent RH; lower levels did not sensitize exposed cells enough, whereas higher levels introduced a dilution effect on ETO gradient to critical cellular site (s). For S. faecalis at dry, 35, 45, 55 and 65 percent RH; D values were ∞, 154, 23, 17 and 6 sec respectively. Optimum percent RH was the highest level tested. Due to certain apparatus limitation, it was difficult to attain higher percent RH levels. Problem has been corrected and further determinations are currently being carried out. Effect of preconditioned cellular water activity level upon consequent optimum

46. Cont'd

sterilant humidity level was negligible under present test conditions. The only effect observed was slight delay in initial lag when test populations were preconditioned to higher a_w levels. This, again, is most likely due to a dilution effect on ETO gradient to critical cellular site(s). It is expected that different cellular types and forms would respond differently. Effect of contaminated material and its bulk/vapor ratio remains undetermined.

47. KINETICS OF BACTERICIDAL ACTIVITY OF ETHYLENE OXIDE IN THE VAPOR PHASE. III. EFFECT OF STERILANT TEMPERATURE AND PRESSURE

El-Bisi, H. M., Vondell, R. M., Esselen, W. B.

Vapor death rates (VDR) were established for desiccant-dry B. subtilis, exposed to ethylene oxide vapor (ETO) at 5 psig; 50, 60, 70 and 80 C; dry and 45 percent RH level. In dry ETO, D values were 408, 312, 205 and 113 sec respectively. at 45 percent RH, D values were 58, 38, 24 sec respectively. It was difficult to provide same percent RH at 80 C due to the apparatus cold-terminal limitation previously reported. In both dry and 45 percent RH ETO the Q_{10} was nearly constant and averaged 1.5, corresponding to z value of about 100 F. Other thermodynamic expressions (E, ΔH, ΔF and ΔS) were computed. Q_{10} is markedly lower than that normally encountered in death by moist heat and atypical of protein denaturation. VDR determinations were repeated at same temperatures but at different percent RH levels, predesignated to provide a constant partial water vapor pressure, namely, that corresponding to 45 percent RH at 60 C. It was evident that percent RH, rather than absolute partial water vapor pressure, governed death kinetics; D values at 50, 60,and 70 C, were 97, 38 and 38 sec

47. Cont'd

respectively. At 50 C higher percent RH caused further decrease in VDR, whereas at 70 C temperature effect was counterbalanced by suboptimum percent RH effect. To explore the effect of sterilant pressure, desiccant-dry B. subtilis spore populations were exposed to ETO at 60 C; dry, 35 and 45 percent RH; 15, 20 and 25 psia. Highest VDR remained attainable at 45 percent RH; D values were 58, 38 and 36 sec respectively. Increase in sterilant pressure seems to accelerate the VDR at exponentially diminishing rate, or perhaps levels off at a certain critical total pressure value.

48. A SELF-STERILIZING COATING FOR SPACECRAFT SURFACES

Willard, M., Alexander, A.

Nature, v. 202, pp. 658-659, 1964

Sterilization techniques chosen for the Surveyor spacecraft include heat sterilization to achieve internal sterility, and gas sterilization with 12 percent ethylene oxide - 88 percent Freon 12 to achieve external sterility prior to launching. Certain Surveyor surfaces require a thermal control coating subsequent to heat sterilization which might introduce embedded viable organisms. The gas sterilant does not penetrate the coating sufficiently to sterilize the surfaces. A self-sterilizing coating was prepared by adding 3.7 percent formaldehyde to the potassium silicate-aluminum silicate coating formulation. Aluminum coupons were inoculated with 10^7 Bacillus subtilis spores, dried in air and painted with 0.1 ml of the formaldehyde-coating mixture. The coupons were dried at 37 C for 24 hr and the coating removed by ultrasonic scrubbing in distilled water. Dilutions were plated in tryptone glucose extract agar, and the poured plates were incubated at 37 C for 19 days. Colony counts were made daily after 48 hr incubation. The sterilant activity of three paint samples was determined weekly for four weeks after preparation. Viable organisms were not recovered at all with one paint sample.

48. Cont'd

Coupons exposed to fresh mixtures of the other two paint samples had 10-150 viable spores, but survivors were not detected in all subsequent trials. Colonies first appeared after 14 days incubation in tests which showed survivors.

49. ENCAPSULATION PROCESS STERILIZES AND PRESERVES SURGICAL INSTRUMENTS

Montgomery, L. C., Morelli, F. A.

NASA Tech Brief 64-10066, July 1964

A possible solution to the problem of sterilizing surgical instruments in an encapsulating material that will maintain a sterile condition indefinitely is described. Steam autoclaving is not required, and the encapsulating material is readily removable. Ethylene oxide gas or liquid is mixed with a suitable plasticizer and uncured polymer that do not react with ethylene oxide. Instruments are dipped into a solution of the mixture and removed for vacuum degassing and curing of the adherent film. Instrument sterilization occurs during the degassing and curing process during which all instrument surfaces are exposed to the sterilizing action of the released ethylene oxide. A residual quantity of the sterilant remains diffused within the adherent solid polymer film. This process developed from NASA's search for methods of sterilizing and protecting space hardware against microorganisms.

50. ALTERATIONS IN SENSITIVITY TO DAMAGING AGENTS BY CULTURAL CONDITIONS IN ESCHERICHIA COLI

Stapleton, G. E., Engel, M. S., Orce, L. V.

Bacteriological Proceedings, p. 60, 1962

It has been demonstrated that conditions that occur during the growth cycle of several strains of Escherichia coli determine the sensitivity of the resulting cell population to inactivation by radiation, heat, and toxic chemical agents. It is known that the alterations in radioresistance thus observed are the result of a complex interaction of a number of known and unknown factors. Although the development of resistance occurs late in the stationary phase of the culture cycle, after cell division is reduced, cell division is required to alter the sensitivity of the population within short-term incubation periods. An investigation of the response of irradiated resistant and sensitive populations of the B/r strain to simple and complex media indicates that the expression of resistance is dependent on post-irradiation nutritional supplementation. The differential medium effect for the sensitive and resistant cells suggest that radioresistance reflects the relative capacity of the two types of cells to repair damage incurred by the irradiation. The expression of radiation-induced mutation at several loci in

50. Cont'd

resistant and sensitive populations of cells is being investigated as a necessary part of the evaluation of the sites involved in the repair process.

51. THE REHYDRATION OF DRIED BACTERIA

Cabelli, V. J.

Bacteriological Proceedings, p. 125, 1962

An investigation was conducted into the mechanisms by which aerosolized bacteria are rendered non-recoverable upon rehydration. The survival of aerosolized Pasteurella tularensis, strain Schu, over a 24 hr period immediately following collection in the all-glass impinger was used as the test condition. L-cysteine and phosphate buffer mixtures (pH 7.0) markedly increased the survival of rehydrated P. tularensis. This effect was found to be specific and not a consequence of the anti-oxidant properties of the cysteine or the buffering capacity of the phosphates. The effectiveness of various monosaccharides and their polyhydric alcohol derivatives, when present by themselves or in combination with i-inositol or sucrose, appeared to depend on the length of the carbon chain. Marked improvement in the survival of the organisms in solutions, of amylose, soluble starch or glycogen but not inulin or dextrin, was observed when inositol also was included in the collecting fluid. Of the carbohydrates studied, melezitose appeared to be the most efficatious for the survival of rehydrated P. tularensis. The differences observed between the various carbohydrates were largely

51. Cont'd

negated when they were tested in the presence of optimum concentrations of inorganic phosphate and cysteine. The loss from the cell of essential metabolites because of an altered cell permeability resulting from aerosolization is postulated as a major factor in the death of the micro-organisms on rehydration. It is suggested that the various carbohydrates are effective by decreasing the permeability of the cell wall or cell membrane and by reducing the osmotic gradient of the cell to its environment.

52. SURVIVAL OF ESCHERICHIA COLI FROZEN IN CELL EXTRACTS

Ambrosini, R. A., Bretz, H. W.

Bacteriological Proceedings, p. 6, 1963

Extracts of 10^9 to 10^{10} washed Escherichia coli cells/ml prepared by freezing and thawing, by boiling, or by autoclaving yielded a protective factor extract (PFE) for the increased survival of 2 X 10^8 E. coli test cells/ml when frozen at -70 C for 5 min and stored at -9 C for 7 days. Tubes containing 5 ml were thawed 3 min at 37 C and viability assessed by agar smear plates after dilution in buffer. Cells in PFE averaged 61 percent survival in 15 expts compared to 30 percent in 0.067 M Sorensen's phosphate buffer pH 7. Dilution of concentrated PFE or decreasing the number of cells from which PFE was extracted decreased survival. PFE was adsorbed by cells as shown by increased survival of such pretreated cells when used as test cells and by a decrease of protectability of the supernatant after adsorption. PF is probably not a metal or small molecule as shown by ashing, chelation, and dialysis expts. Survival was correlated with the carbohydrate content of PFE but preliminary attempts to purify the material by fractionation procedures have given anomalous results. Norite treatment or dialysis of PFE increased its activity slightly indicating that cell

52. Cont'd

extracts contain both large mol wt protective and small mol wt inhibitory substances. The relative proportions of these two materials in the cells could account for some of the great variability in frozen storage studies.

53. GERMINATION OF SPORES AS A CONSEQUENCE OF AEROSOLIZATION AND COLLECTION

Levin, M. A., Cabelli, V. J.

Bacteriological Proceedings, p. 26, 1963

In the course of studies on the applicability of Bacillus subtilis (var niger) spores to function as a tracer for the physical behavior of biological aerosols, it was observed that the "spores" were sensitive to stresses imposed by dissemination and collection in liquid impingers. This was manifested by decreases in viable recovery as a function of the collecting device. Germination during the collection process was shown by the increased release of dipicolinic acid, changes in staining reactions, and heat susceptibility with collection time. The dependency of germination on the O_2 content of the air sampled was shown by the absence of these manifestations when N_2 was used. The absence of a requirement for an exogenous source of nutrients was demonstrated by the manifestation of these effects even though the heat shocked spore suspensions were suspended or collected in distilled water. Protection of the spores during the collection processes was accomplished by the addition of skim milk solids in the impinger fluid; of the skim milk fractions studied, only lactose was effective and its protective effect was

53. Cont'd

augmented by soluble starch. Evidence was obtained to show that lactose, but not several other sugars tested, was effective by virtue of a protective action on the germinated spore. The mechanism by which lactose protects the cells was demonstrated.

54. SURVIVAL OF FREEZE-DRIED *SERRATIA MARCESCENS* AS A FUNCTION OF AGE

Dimmick, R. L., Dunn, S. A.

Bacteriological Proceedings, p. 47, 1963

If death rates are to be used to correlate two phenomena (e.g. death during storage in the dry state and "spontaneous" free-radical production (Dimmick, *et al*, 1961, Nature *192*, 776) it is important to know whether the rates are exponential. Samples of a growing culture, obtained 1, 2,3,5,7,11,15,18,21, and 24 hr after inoculation, were lyophylized without additives. Samples were frozen at temperatures of -20 and -65 C, and both mineral salts with glycerol and trypticase soy agar were used as plating media after cultures were reconstituted. Results of storing cells for 15 days under vacuum at 22 C were plotted as survivor curves, and the curves were assembled to form 3-dimensional models. We found: (i) some curves appeared to be logarithmic, others were not, depending upon culture age; (ii) when initially frozen at -20 C, cells in the growth phase survived storage better than when frozen at -65 C; during the stationary phase survival was equal, whereas, after 24 hr growth, cells survived storage after -65 C pre-treatment better than -20 C; (iii) there was a rhythmic function of both culture age and

54. Cont'd

storage time - neither medium was "best". We concluded that a rapid and alternating response to subsequent freeze-drying is induced in cells in a growing culture, similar to the induced response to thermal stress (Dimmick, 1961, Bacteriol. Proc., p. 57), and that the question of whether or not bacteria die logarithmically, or by any random process, cannot be answered until a better criterion for viability is available.

55. EFFECT OF SODIUM CHLORIDE AND pH ON THE OUTGROWTH OF SPORES OF TYPE E *CLOSTRIDIUM BOTULINUM* AT OPTIMAL AND SUBOPTIMAL TEMPERATURES

Segner, W. P., Schmidt, C. F., Boltz, J. K.

Bacteriological Proceedings, p. 3, 1964

The determination of the minimal pH permitting outgrowth of spore inocula of four strains of Type E *C. botulinum* in a medium containing 5 percent trypticase, 0.5 percent peptone, and 0.4 percent glucose at optimal and suboptimal temperatures is affected by the reducing agent present in the system. When the reducing agent was sodium thioglycolate at 0.02 percent concentration or L-cysteine hydrochloride at 0.05 percent, outgrowth occurred at 85 or 46 F at substantially lower pH values than when sodium thioglycolate was present at a 0.2 percent concentration. At 85 F, one strain gave outgrowth at pH 5.2 and 5.0, while three other strains gave outgrowth at pH 5.4 but not at 5.2 and 5.0. At 46 F, spores of the four strains tested showed outgrowth at pH 5.9 but not at 5.7. The salt concentration required for prolonged inhibition of outgrowth of spores of the same four strains is considerably lower at 46 and 50 than at 70 and 85 F. Salt concentrations slightly lower than those providing inhibition tend to extend the incubation time required for growth to

55. Cont'd

appear. Salt concentrations required for prolonged outgrowth may be interrelated with spore inoculum level and medium composition.

56. LONGEVITY OF ANAEROBIC SPOREFORMING BACTERIA PRESERVED IN LYOPHIL AND IN SOIL

Haynes, W. C., Rhodes, L. J.

Bacteriological Proceedings, p. 37, 1964

Although lyophilization has proven reliable for long-term preservation of many bacteria, little has been reported regarding the longevity of lyophilized anaerobic sporeformers. For some of them, preservation in sterilized soil has been effective, but again little has been reported about longevity. In the ARS Culture Collection, 26 strains comprising 9 species, most of them of industrial importance, were preserved by lyophilization and by drying in soil. To hedge against possible loss of cultures, lyophil tubes and soils were replicated and prepared anew from time to time. Soil cultures ranging in age up to 20 years and lyophil preparations more than 13 years old were checked for viability every few years. All lyophil tubes of 25 strains were viable every time they were tested. One strain was erratic, sometimes surviving as long as 3 years, but never longer. Soil cultures were less consistent. Of several tubes of a given age tested at once, some usually are viable, some non-viable, and some viable only if heat-shocking is not performed. However, one or more soil cultures of each of the 26 strains continued viable

56. Cont'd

throughout the observation period. Greatest longevity observed exceeds 13 years in lyophil and 20 years in soil. This variation in longevity may be related to spore concentration of the inoculum, uniformity of distribution of the inoculum, and type of soil used.

57. INFLUENCE OF GROWTH MEDIUM AND STORAGE AT 4 C ON DEATH OF AIRBORNE SERRATIA MARCESCENS BY SORPTION OF WATER

Hatch, M. T., Dimmick, R. L.

Bacteriological Proceedings, p. 113, 1964

Investigators attribute death of airborne bacteria to an alteration in protein structure resulting from cellular dehydration. Cells of S. marcescens, grown in Trypticase Soy Broth (TC), were diluted in the growth medium and atomized into the air at a relative humidity (RH) of 22 percent. The death rate increased when we suddenly raised the RH to 54 percent. This "sorbed death" phenomenon was not demonstrable with airborne cells subjected to an increase in RH from 59 to 72 percent. Death from the sorption of water also was not apparent with cells grown and atomized in a modified Bunting's broth (BB). Cells grown in BB and atomized in TC demonstrated an increased death rate only if the cells were in an air atmosphere where the RH was suddenly raised. If the RH was held constant or lowered, no increased death rate was observed. Conversely, a decreased death rate, after an increase in RH, was evident with cells grown in TC and atomized in BB. Thus, TC appeared to be "toxic" and BB "protective". However, cells aged by prolonged storage at 4 C in BB and then suspended in cold TC for 30 min became increasingly resistant to death by sorption of water as a function of the initial

57. Cont'd

storage period. Evidently, death was dependent upon pre-aerosol treatment. Moreover, death was not entirely attributable to toxic substances in the growth or spray media, or to dehydration of protein, because death by sorption of water was correlated with the metabolic activity of the cells. We believe that an imbalance in metabolic function caused the airborne cells to die by sorption of water.

58. INFLUENCE OF STORAGE, AEROSOLIZATION, AND REHYDRATION ON THE PERMEABILITY OF PASTEURELLA TULARENSIS

Hayes, D. K., Cabelli, V. J.

Bacteriological Proceedings, p. 114, 1964

After being subjected to a variety of stresses, P. tularensis appears to lose in part the ability to maintain a constant internal environment. This is manifested by a decreased ability to retain intracellular constituents such as inorganic phosphate and is related to a decrease in the percentage of culturable organisms. Aerosolized cells which are rehydrated and held in gelatin saline solutions demonstrate these effects. However, cells rehydrated and held in either a sucrose-inositol solution or a solution containing in addition phosphate, thiourea, cysteine, and spermidine maintain intracellular inorganic phosphate levels and are more readily culturable. In aged cells, the capacity of retaining inorganic phosphate in both sucrose-inositol and gelatin-saline solutions is decreased. In the more complex fluid which contains phosphate, intracellular inorganic phosphate is maintained after stress. Under the environmental conditions which favor loss of orthophosphate by the cell, some inorganic phosphate is maintained at the expense of organic phosphates. The mechanisms by which certain organic phosphates are hydrolyzed

58. Cont'd

to give rise to inorganic phosphates have been considered. The rate of phosphate loss in a low phosphate environment is a function of temperature. The data suggest that at 37 C more than one process is occurring.

59. AN ELECTROCHEMICAL METHOD FOR THE STERILIZATION OF OBJECTS CARRYING SPORES OF MICROORGANISMS

Rechmenskii, S. S.

Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (USSR), No. 7-8, pp. 152-155, 1939 (In Russian)

(Abstracted in Chemical Abstracts, v. 36, p. 4850^2)

Electric current activated the sterilizing action of mercury preparations on spores of Bacillus anthracis. Sterilization is effected in a shorter time or at a lower concentration of the sterilizing agent.

60. EFFECTS OF SHOCK, VIBRATION, AND ACCELERATION ON THE CIRCADIAN GROWTH RHYTHM OF NEUROSPORA CRASSA

LaRochelle, M. F., Berliner, M. D.

Bacteriological Proceedings, p. 112, 1964

N. crassa with a daily rhythmic growth pattern was subjected to a series of simulated missile launch and reentry conditions. Ring formation frequency and growth rate as determined by ring amplitude were recorded after tests. These serve as indicators of changes in the organism's endogenous circadian rhythm. Replicate groups were inoculated 48 and 24 hr and immediately prior to environmental testing. Response to individual tests was as follows. (i) Shock (100 g's 6 m/sec, 1/2 Sine pulse, one drop): All cultures had a slightly accelerated ring formation averaging 21 hr; most often in cultures inoculated 24 hr prior to testing. (ii) Random vibration (a) (Flat spectrum, 20 to 2,000 cps, 0.07 g^2/cps for 10 min): Vibration produced no noticable effect on cultures inoculated 24 hr before test. Ring formation was slightly accelerated or inhibited on other groups. (b) (Flat spectrum, 10 to 2,000 cps for 3 min): Although the vibration was more intense than (a), cultures inoculated 24 hr before test maintained a constant and expected ring formation. (iii) Acceleration (17 g's for 5 min): Only cultures

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started immediately prior to testing had marked accelerated ring formation. (iv) Cultures subjected consecutively to all environments indicated that N. crassa will successfully withstand anticipated BIOS launch conditions without serious interruption of its circadian rhythm.

61. EFFECTS OF MAGNETIC FIELDS ON GROWTH OF HELA CELLS IN TISSUE CULTURE

Halpern, M. H., Greene, A. E.

Nature, v. 202, no. 4933, p. 717, 1964

Growth of HeLa cells in tissue culture is not inhibited or otherwise obviously affected after exposure to a 1,200-gauss magnetic field.

62. TEMPERATURE COEFFICIENTS OF THE RATE OF INJURY BY HEAT (Extract from table LXII (p. 174); various authorities)

Bělehrádek, J.

Temperature and Living Matter, Verlag von Gebrüder Borntraeger, Berlin, 1935

ORGANISMS	Q_{10}	TEMPERATURE RANGE (C)
SPORES		
B. anthracis	10.7	90.4 - 105.3
Various bacteria	5.6	100 - 140
C. botulinum	9.5	100 - 115
B. subtilis	4.4	110 - 140
B. robur	6.0	110 - 130
B. anthracis, dry	2.9	120 - 135
B. anthracis, moist	3.8	90 - 105
C. tetani, dry	2.0	95 - 105
C. tetani, moist	7.1	125 - 135
BACTERIA		
B. coli commune	12	48.4 - 52.7
B. typhosum	136	49 - 59
S. pyogenes aureus	29	48.4 - 52.4

63. THERMAL RESISTANCE OF CERTAIN DOMINANT AEROBIC BACILLI

Iyer, V., Bhat, J. V.

Journal of Scientific and Industrial Research (India), v. 11B, pp. 427-430, 1952

The thermal resistance of spores of four strains of bacilli known to possess high resistance was determined. Bacillus cereus (B1) and B. subtilis (B13) were isolated from Bombay air, B. megaterium (Sd) from commercial sugar, and B. subtilis (Ma) from a can of mangoes showing gaseous spoilage. Spores were harvested from a peptone-beef extract agar medium, shaken with glass beads, filtered and assayed. One-ml portions containing 10^5 spores were sealed in 6-ml ampoules. Ampoules were heated over the range 100-120 C for four exposure periods at each 5-degree interval in water and castor oil baths. Preliminary experiments established suitable exposure times at each temperature. Ampoules were cooled rapidly after heating, and 4-ml nutrient broth added. The resealed ampoules were incubated at room temperature for up to 20 days and examined at intervals for growth. Six ampoules of each organism were subjected to the same temperature-time relationship. B. subtilis (B13) was most resistant. Survivors were observed after 60 min at 120 C. A summary of the thermal resistance data follows:

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Temp	Calculated Z value (min)			
C	B1	Sd	B13	Ma
100	27.3	26.4	29.1	28.4
105	21.9	20.7	24.7	22.6
110	16.7	14.6	19.9	18.8
115	9.4	8.4	14.6	11.3
120	5.7	4.5	11.3	6.3

Phantom thermal death time curves were plotted for process calculations in which the objective is pasteurization or sterilization of a product.

64. APPARATUS FOR DETERMINING THE NUMBER OF MICROBES IN THE AIR

Zampach, A.

Czechoslovak Patent No. 93066, Dec. 15, 1959

Nov. 29, 1962

JPRS: 16,407 (OTS)

A revolving cylindrical drum device is described which takes in air by means of an air pump and deposits micro-organisms on plastic bands covered with 2 mm of solid growth medium. The plastic bands are sterilized by dry heat at 140 C for 3 hours. The drum is sterilized by means of an antiseptic such as mercury phenylborate. The rotation speed is set to the appropriate intake capacity which may vary from 0.25 to 3 liters per minute.

65. THE EFFECT OF HEAT AND IRRADIATION ON THE MICROFLORA OF CANNED HAMS

Drake, S. D., Evans, J. B., Niven, C. F. Jr.

Food Research, v. 25, no. 2, pp. 270-278, 1960

The radiation resistance of the microflora in canned hams which had been or had not been heat-processed, and the heat and radiation resistance of pure cultures of isolated bacteria were investigated. Heated hams had been processed to a core temperature of at least 150 F (66 C). Hams were frozen at -34 C and irradiated with approximately 1 megarad gamma rays. The hams were cold at the conclusion of the 24 hr irradiation period. Raw, non-irradiated canned hams had counts ranging from 47×10^4 to 67×10^7 per g in surface samples, and 24×10^3 to 58×10^6 bacteria per g in the boned area. Only one of the eight hams of this type irradiated and incubated at 37 C for one month showed bacterial growth. This ham contained 2×10^8 enterococci per g. Eight heat processed, non-irradiated hams were incubated, and had counts ranging from zero (2 samples) to 76×10^7 per g. Three heat processed irradiated hams showed no growth, but another contained homofermentative lactobacilli, and four had moderate numbers of bacilli in total aerobic counts ranging from 12×10^3 to 30×10^5 per g.

65. Cont'd

The LD_{99} of spores from several Bacillus isolated were: B. licheniformis, 249,000 rads; B. coagulans, 338,000 rads; B. cereus, 513,000 rads. Streptococcus faecium strain HS-5 survived 130 min at 145 F (63 C) and 55 min at 150 F (66 C). The radiation resistance was as high as that of most bacterial spores. There is no direct correlation between heat resistance and radiation resistance. The effect of combined heat and irradiation was investigated with S. faecium strain HS-5. When survivors of a heat treatment that killed 90 percent of the cells were irradiated, the survivor curve had less initial lag, but the curve generally paralleled that obtained with unheated cells. Suspensions initially irradiated to kill 90 percent of the cells were slightly more heat sensitive than were unirradiated cells. The main change in the survivor curve again was elimination of the initial lag phase. Sodium chloride (5 percent) in the suspending medium appeared to sensitize the cells to both heat and irradiation.

66. STUDY OF HEATED BONEMEAL CONTAMINATED WITH BACILLUS ANTHRACIS

Wende, R. D., Burdon, D. L.

Bacteriological Proceedings, p. 43, 1960

Bonemeal has been found an especially favorable material for survival of anthrax spores, despite the common presence of an abundant and varied saprophytic flora. The procedures necessary to sterilize bonemeal by dry heat were investigated. Portions of raw and steam-sterilized bonemeal were contaminated with known numbers of virulent anthrax spores. A systematic series of test with 25 g and 10 g amounts of the contaminated bonemeal contained in small metal pillboxes was carried out by heating the pillboxes at 120-160 C for periods of 25 to 65 min in a dry heat oven. Heating temperatures and times were controlled by a Honeywell temperature recorder. Complete sterilization of the 25 g samples was attained after 160 C for 60 min and for the 10 g amounts after 160 C for 40 min. Of special interest was the repeated recovery of previously undescribed variants of Bacillus anthracis from bonemeal samples heated at temperatures and times short of complete sterilization. Strains isolated after moderate heat exposure were virulent, but attenuated, while those recovered after further heating were completely

66. Cont'd

nonvirulent. Changes in the cellular and colonial morphology of the variants increased with the degree of heat exposure. All the variants retained characteristic biochemical reactions of B. anthracis and remained distinguishable from B. cereus. None of the avirulent mutants tested immunized mice against virulent anthrax spores. The regularity with which these variant cultures could be produced at will suggests the probable general usefulness of this heating procedure for the study of variation in sporeforming bacilli.

67. THE INFLUENCE OF SPORULATION TEMPERATURE ON THE THERMAL RESISTANCE AND CHEMICAL COMPOSITION OF ENDOSPORES

Lechowich, R. V., Ordal, Z. J.

Bacteriological Proceedings, pp. 44-45, 1960

It has been demostrated that the incubation temperature at which endospores are produced has an effect on their thermal resistance. Also, the unique presence of dipicolinic acid (DPA) in bacterial spores suggests that it is associated with thermal resistance. It has been further postulated that cations are likewise intimately associated with the thermal resistance properties of the endospores. This investigation was performed in order to elucidate a possible relationship between DPA and cations and the endospores relative resistance to thermal destruction. The thermal resistance of spores of Bacillus subtilis was varied by growth at two sporulation temperatures and their cation contents determined by means of the emission spectrograph. The medium used for spore production was a modified thermoacidurans agar, pH 6.8, which contained 1 ppm manganous sulfate. The thermal sporulation temperature increased from 30 to 45 C. Spore crops of B. coagulans were produced at incubation temperatures of 30, 45, and 52 C. The thermal resistance of these spore

67. Cont'd

crops increased as the sporulation temperature became greater. Analyses of these spore crops indicated that they contained similar amounts of cations, while the DPA content decreased as the sporulation temperature increased. The ratios of millimoles of cations to millimoles of DPA per 1 X 10^{10} spores were calculated for each spore crop of both organisms. The ratios were found to increase directly with increased thermal resistance. The implications of these and other data will be discussed.

68. STEAM STERILIZATION AND DISTILLATION OF POISON HELP PROTECT COOLING TOWER WOOD FROM BIOLOGICAL ATTACK

Regutti, C. W., Power, T. H.

Materials Protection, v. 1, no. 10, pp. 84-85, 1962 (Abstracted in Chemical Abstracts, v. 58, p. 10671c, 1963)

Details are given of a method for sterilizing cooling tower wood infected with fungi and bacteria. Tests on a cell at a large oil refinery showed that 2 hours at 145-160 F was sufficient to kill fungi even in large timbers. A phenolic bactericide distilled into the cell and allowed to crystallize on the sterilized wood is believed adequate to retard future attack by biological aggressors.

69. RESISTANCE OF DRY BACTERIAL SPORES TO STERILIZATION BY MOIST AND DRY HEAT

Koesterer, M. G., Bruch, C. W.

Bacteriological Proceedings, p. 30, 1962

The comparative resistance of dry spores of Bacillus subtilis var. niger and B. stearothermophilus strain 1518 to destruction by moist and dry heat was investigated. Washed spore preparations were inoculated onto filter paper strips and air-dried. Lots of strips were prepared to yield specific concentrations in the range of 1-10 X 10^5 spores. Exposure of the strips in partially covered test tubes in an autoclave yielded F values (time for total kill) at 121 C of 15 min or greater for both species. To eliminate the discrepancies from air entrapped by this procedure, the strips were exposed in envelopes. The F value for B. subtilis var. niger at 110 C was less than 5 min, while spores (10 X 10^5) of B. stearothermophilus had an F value of 20 min at 121 C. In the dry heat studies the strips were exposed in test tubes in heated metal blocks. Thermal death time curves from 121 C to 170 C were prepared. The spores of B. subtilis var.niger were 3.5 to 5 times more resistant than the spores of B. stearothermophilus to destruction by dry heat. D values (time for destruction of one log of spores) at 121 C were

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47.5 min for B. subtilis var. niger and 14.3 min for B. stearothermophilus and at 160 C the D values were approximately 1.6 min and 0.3 min respectively. The resistance of bacterial spores to destruction by moist or dry heat involves factors other than resistance to increased temperature.

70. STUDIES ON DRY HEAT FOR THE STERILIZATION OF ELECTRONIC COMPONENTS OF ASTROBIOLOGICAL SPACE PROBES

Bruch, C. W., Koesterer, M. G., Bruch, M. R.

Bacteriological Proceedings, p. 31, 1962

Studies of extraterrestrial biology would be jeopardized or even made forever impossible, by inadvertent contamination of celestial bodies with earth flora. The prevention of contamination from disintegration of components of space probes requires sterilization of these components before assembly of the probes. The development of adequate dry-heat sterilizing cycles must be based on organisms with high dry-heat resistance. Screening studies with air heated to 125 C were carried out for air-dried spores from several species of the genus Bacillus and from the PA 3679 and Vera strains of Clostridium sporogenes. Several strains of B. subtilis were found to be highly resistant to dry heat sterilization. Six different lots of soil were exposed to dry heat at this temperature. Although the soil samples were several fold more resistant to dry heat than were the preparations of spores dried on filter paper strips, mesophilic sporeformers isolated from these heated soil samples had levels of resistance similar to those found for B. subtilis. The use of various carriers for these spores showed that preparations dried on

70. Cont'd

paper strips or in test tubes were easier to kill than were preparations dried on sand or vermiculite. These latter preparations had levels of resistance approaching those found for the soil samples. The resistance of spores to dry heat involves various factors which must be elucidated before dry heat cycles for electronic components can be developed.

71. SURVIVAL OF SPORES AT SEVERAL TEMPERATURES IN ULTRAHIGH VACUUM

Davis, N. S., Silverman, G. J., Goldblith, S. A., Keller, W. H.

Bacteriological Proceedings, p. 31, 1962

Spores of Bacillus megaterium, B. subtilis var. niger, B. stearothermophilus, Clostridium sporogenes, and Aspergillus niger were maintained at pressures of 10^{-8} to 10^{-9} mm Hg for four days at constant temperatures which ranged from -110 C to +83 C. B. stearothermophilus was the only organism which showed a significant decrease in viability in ultrahigh vacuum at -110 C and +25 C. The viability of B. megaterium was reduced to 2.6 percent at 53 C while less than 1 percent of C. sporogenes survived. On the other hand, 63 percent of the B. subtilis var. niger spores were resistant at 53 C in vacuum. A. niger spores withstood 60 C in vacuum better than the spores of all but B. subtilis var. niger. The combined action of heat and vacuum was clearly greater than that of heat alone since survival of B. stearothermophilus and B. subtilis var. niger was appreciably lower at 60 C in vacuum than at 60 C and atmospheric pressure. An initial experiment at elevated temperature demonstrated that only spores of B. subtilis var. niger (0.0007 percent) and A. niger (0.2 percent)

71. Cont'd

survived 88 C in vacuum. Efforts to improve viability by altering the diluent and rehydration conditions have not been successful to date.

72. EFFECT OF RADIATION ENVIRONMENT ON THE THERMAL RESISTANCE OF IRRADIATED SPORES OF CLOSTRIDIUM SPOROGENES P.A. 3679

Licciardello, J. J., Nickerson, J. T. R.

Journal of Food Science, v. 27, no. 3, pp. 211-218, 1962

Thermal resistance at 100 C was determined after the spores had been irradiated in air, vacuum (1 mm Hg), or nitrogen with gamma or cathode rays while suspended in phosphate buffer, pH 7.0, nutrient broth, or ham puree. Samples were at 20, -78 C, and 66 - 68 C during irradiation. Sealed melting point capillary tubes each containing 0.025 ml of spore suspension were transferred automatically from the heating bath to the cooling bath. The D values of spores in phosphate buffer were not significantly different in air or vacuum for a given radiation dose. Thermal resistance did, however, increase significantly at 660,000 rep in a nitrogen atmosphere. Enhanced lethality in air or vacuum was attributed to greater accumulation of hydrogen peroxide. Combined irradiation - heating was more effective in neutral than in acidic phosphate buffer. Thermal resistance was independent of spore concentration for samples irradiated with less than 660,000 rep, but increased at higher radiation doses, presumably because of protective materials released by dead cells and spores. Z values measured over the interval

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100 - 110 C averaged 14.2 F, and did not differ for spores irradiated and unirradiated prior to heating. Thus irradiation prior to heating changed the F value but not the Z value. Irradiation sensitized spores to heat to a greater degree in phosphate buffer, pH 7.0 than in ham puree or nutrient broth. Thermal resistance in buffer was not significantly different between -78 C and 20 C over a dose range of 0 to 660,000 rep and between 20 and 66 - 68 C with either 165,000 or 300,000 rep. Exposure to 660,000 rep before heating at 66 - 68 C resulted in a marked decrease in heat resistance compared to similar experiments at 20 and -78 C. The irradiation doses at various temperatures required to destroy 90 percent of the spores in phosphate buffer, pH 7.0 were: 171,000 rep at 20 C, 196,000 at 66 - 68 C and 260,000 at -78 C.

73. ALTERATION OF SPORULATION IN ASPERGILLUS NIGER BY HEAT AND DEUTERIUM OXIDE

Henderson, T. R., Dinning, J. S.

Nature, v. 194, no. 4827, pp. 498-499, 1962

Aspergillus niger spores suspended in water, in 99.5 percent deuterium oxide, and in the dry state, were heated in a water or oil bath maintained at 70 and 100 C. Spores in deuterium oxide are 1000 to 10,000 times more resistant to heat inactivation than spores suspended in water. Dry spores are at least 10 to 100 times more resistant than spores in deuterium oxide. Nonsporulating strains and conidiophore variants occurred in cultures prepared from heated spores which had been in deuterium oxide. A probable explanation of the synergistic effect of deuterium oxide and heat in producing sporulation anomalies is an alteration in the hydrogen bonded secondary structure of DNA. Temperatures of 70-100 C have been shown to alter DNA in vitro from a predominantly helical configuration to a random coil structure. The effect of heat on A. niger spores (ca 5 X 10^8 spores per test) in these experiments is summarized in the following table.

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Heating Conditions hr	C	Suspension Medium	Survival %
3	70	water	0.001-0.0001
1	100	water	0.00001
3	100	dry	0.1-1
1-2	100	deuterium oxide	0.01-0.1

74. THERMAL INACTIVATION OF HEAT-RESISTANT BACTERIAL SPORES IN MILK CONCENTRATE

Segner, W. P.

Dissertation Abstracts, v. 23, pp. 1876-1877, 1962

This study was initiated to indicate the ultra-high thermal treatments that might be required for the sterilization of a milk concentrate containing approximately 36 percent total solids. Rates of inactivation of spores of Bacillus coagulans WH-9, B. stearothermophilus 1518, and Clostridium sporogenes (PA 3679) were determined in the 3:1 milk concentrate and in M/15 phosphate buffer at pH 6.2. Pyrex thermal inactivation tubes containing 0.5 ml of a concentration of 10^6 spores per ml were immersed in a bath at lethal temperatures for various periods of time. The number of surviving viable spores was determined by plate counts. Although spore inactivation was predominantly logarithmic, the initial portion of the curves was usually curvilinear. This deviation was attributed to temperature changes during the come-up time rather than evidence that destruction occurred nonlogarithmically. Thermal death time data were established on the basis of six decimal reduction times at three bath temperatures to indicate the lethal process for reduction of spores from 10^6 to one spore per ml regardless

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of the come-up time and cooling lag. Comparison of the F and z values in the following table demonstrate the greater heat resistance of B. stearothermophilus spores.

Spores	Buffer		Milk Concentrate	
	F value (min)	z value (°F)	F value (min)	z value (°F)
B. stearothermophilus	10.33	21.2	11.67	21.9
C. sporogenes	3.38	19.7	4.33	20.3
B. coagulans	1.67	15.6	1.75	16

B. stearothermophilus spores required a high activation temperature. After the spores had been heated rapidly to 120 C and cooled immediately in an ice bath, a tenfold increase in viable spore count was obtained over that after heat-shocking at 100 C. It was necessary to dilute the milk concentrate at least 1:100 to obtain germination of spores surviving the heat treatments since the concentrate inhibited germination of many spores. Aerobic sporeforming bacteria isolated from 23 of approximately 200 lots of processed milk concentrate were concluded to represent poststerilization contaminants.

75. THE SIMULTANEOUS LETHAL EFFECT OF TEMPERATURE AND GAMMA RADIATION ON BACTERIAL SPORES

Graikoski, J. T.

Dissertation, The University of Michigan, Ann Arbor

128 pp., 1961

The role of temperature during irradiation on the subsequent survival of spores was studied. Anaerobic bacterial spores were slightly more resistant to radiation at -70 than at 4 C. The maximum number of these spores survived radiation exposure at a temperature just below the thermal lethal threshold for the particular strain. The lethal threshold for Clostridium botulinum spores was found to be about 85 C. Above this temperature, spores were rapidly inactivated by radiation. The greatest number of survivors of putrefactive anaerobe NCA 3679 spores was observed in the range 90 to 100 C. Progressively greater numbers of survivors were obtained as the temperature was increased above room temperature. Although radiation increases the sensitivity of spores to heat, the thermal lethal temperature is not lowered by this treatment. Spores which exhibit greatest resistance at the various temperatures during irradiation also have greater thermal resistance. Determination of the sterility dose must consider the temperature at which irradiation is carried out. If the sensitization of bacterial spores to heat by

75. Cont'd

radiation is to be utilized, the irradiation must be conducted at the thermal lethal threshold of the organism under consideration. The most thermally resistant organism must be used in the evaluation of this combined method of sterilization. A pre-irradiation treatment, followed by post-irradiation heating at a lethal temperature must be employed to take full advantage of the sensitization of bacterial spores to heat. Heat must be applied simultaneously with radiation in order for the observed temperature effect during irradiation to be demonstrated. Pre-irradiation treatment of spores at a dose which is sporicidal for large numbers of spores does not significantly lower the temperature threshold necessary for thermal inactivation.

124 references

76. SENSITIZING BACTERIAL SPORES TO HEAT BY EXPOSING THEM TO ULTRA-VIOLET LIGHT

Curran, H. R., Evans, F. R.

Journal of Bacteriology, v. 36, pp. 455-465, 1938

Spores of Bacillus albolactis, B. cohaerens and a strain belonging to the B. mesentericus group were found to be more susceptible to heat after exposure to ultraviolet light. In order for the heat sensitization phenomenon to manifest itself, it was necessary for the UV exposure to be of sufficient duration to be sporicidal for a large number of organisms. Shorter wavelengths in the range 350-1600 A were more effective than 2537 A in inducing heat sensitization at 98 C. The most heat resistant organism tested, B. cohaerens was the most readily sensitized to heat by UV.

77. THERMAL RESISTANCE OF MICROORGANISMS TO DRY HEAT; EFFECT OF HEATING MEDIUM

Pheil, C. G., Nicholas, R. C., Pflug, I. J.

Bacteriological Proceedings, p. 5, 1963

Spores of Schmidt's 5230 strain of Bacillus subtilis that had been dried in a vacuum oven were heated in atmospheres of helium, nitrogen, air, oxygen and carbon dioxide in small cups inside thermal death time cans over the temperature range of 121 to 160 C. The heat resistance was determined by end-point techniques.

The results suggest that these gases have a similar but different effect on the heat resistance of this organism. The slopes of the thermal resistance curves are the same (approximately 18 C) for all gases, but the intercepts are different. The order of increasing lethality throughout the temperature range is helium, nitrogen, oxygen, air and carbon dioxide. The gases appear to be grouped: helium and nitrogen similar and oxygen, air and carbon dioxide similar in effect.

It can be concluded from this study that the nature of the dry gas surrounding cells during heating has an affect on the destruction rate of spores of B. subtilis.

78. INDUCED GROWTH OF ARTHROBACTER CITREUS AT ELEVATED TEMPERATURES BY ASSOCIATED MICROORGANISMS

Johnson, M. B., Chan, E. C. S.

Bacteriological Proceedings, p. 44, 1963

The arthrobacters are described as typical soil organisms that show little or no growth at 37 C (Bergey's Manual). Recent studies in our laboratory show that A. globiformis strains 425 and 8602 would grow at 25, 30, and 37 C on B. B. L. nutrient agar and trypicase soy agar; but the growth of A. citreus 837 above 25 C was found to be a function of associated microorganisms as well as of cultural conditions. On trypicase soy agar A. citreus would grow well at all 3 temperatures. On nutrient agar it would grow at 25 C while at 30 and 37 C it would only grow in the presence of another organism as evidenced by the formation of satellite colonies. Such associated microbes included Aerobacter aerogenes, Pseudomonas sp., Bacillus cereus, Streptomyces scabies, Penicillium notatum, Aspergillus niger, Saccharomyces cerevisiae and 14 other organisms as well as any air contaminants. Organisms that did not grow at 37 C and consequently not inducing growth of A. citreus were Azotobacter chroococcum, Streptococcus lactis and S. faecalis; however, their growth at 30 C did elicit response from A. citreus. Apparently,

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any organism capable of growing would induce growth of A. citreus. Such induced growth was not carried indefinitely in serial transfer at 37 C. Auxanographic testing of 21 amino acids, 8 peptides, 12 growth factors, 9 purines and pyrimidines, as well as various fatty acids and other biochemicals proved to be negative for growth induction. The effect of associated microorganisms on temperature growth response of A. citreus appears to reaffirm the limitations of physiological pure culture studies as a direct reflection of organism behavior in nature.

79. VIABILITY OF LYOPHILIZED ORGANISMS AFTER AEROSOLIZATION INTO HEATED AIR STREAMS

Greene, V. W.

Bacteriological Proceedings, p. 162, 1963

Lyophilized suspensions of Serratia marcescens (SM) and spores of Bacillus subtilis var niger (BG) were aerosolized in a miniature wind tunnel and exposed to temperatures ranging from 75 C to 200 C for periods approximating one second. Significant inactivation of SM did not occur below 100 C and exposures of 150 C X 1.68 sec to 170 C X 0.5 sec were necessary to effect 99.9 percent kill. Inactivation of BG did not occur below 150 C and exposures of 160 C X 1.68 sec to 200 C X 0.5 sec were necessary to effect 50 percent kill. The thermal-death-time curves were characteristic of multicellular clumps. Small but consistent protective effects were noted when the lyophilized suspensions were coated with a colloidal silica powder before aerosolization.

80. THERMAL DESTRUCTION OF AEROBIC SPORES IN VARIOUS BUFFER SOLUTIONS

Walker, H. W., Matches, J. R.

Bacteriological Proceedings, p. 5, 1963

Thermal resistance of spores is considered to be at a maximum near neutrality. In general, the more acid the pH, the lower the heat required for inactivation. However, in view of some conflicting reports on the influence of acid and alkaline environments, a study was undertaken to determine the influence of buffers of known composition and pH on thermal destruction of spores of Bacillus megaterium 1A28 and B. polymyxa 1A39. Spore suspensions containing approximately 1.0×10^9 spores/ml were heated at 100 C in phosphate, phthalate, borate and citrate buffers adjusted to pH values of 5.5, 7.0 and 8.0. Samples were taken at 3 min intervals and assayed for surviving spores and for release of dipicolinic acid, nitrogen and carbohydrate. Acid and alkaline buffers did not always cause a greater reduction in viable spores than did the neutral buffers. B. megaterium 1A28 showed greater resistance in phosphate buffers of pH 5.5 and 8.0 than in phosphate buffer of pH 7.0. B. polymyxa, however, showed greater stability in phosphate buffers of pH 5.5 and 7.0 and less stability in citrate buffer of pH 8.0 and

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phthalate buffer of pH 5.5. Citrate buffers seemed to be least favorable for survival of spores. In general, dipicolinic acid was released most rapidly and to the greatest extent from those suspensions in which inactivation was greatest. Similar relationships for carbohydrate and nitrogen release were not as apparent.

81. COMBINED EFFECTS OF ULTRAHIGH VACUUM AND TEMPERATURE ON THE VIABILITY OF SOME SPORES AND SOIL ORGANISMS

Davis, N. S., Silverman, G. J., Keller, W. H.

Applied Microbiology, v. 11, no. 3, pp. 202-210, 1963

Considerably fewer spores of Bacillus stearothermophilus, B. megaterium, and Clostridium sporogenes were recovered than were spores of B. subtilis var. niger, and Aspergillus niger after 4 to 5 days at 53 and 60 C in ultrahigh vacuum. There were no significant differences in the recoveries of these five organisms at 25 C and atmospheric pressure, and after exposure to 25 and -190 C in vacuum. At 60 C, a far greater decrease in viability was demonstrated for B. stearothermophilus, B. megaterium, and C. sporogenes in ultrahigh vacuum than at atmospheric pressure. Viable B. subtilis var. niger spores were not detected in an initial 10^7 spores after retention at 90 C and ultrahigh vacuum, and 10^4 spores were viable after 5 days at 90 C and atmospheric pressure from a initial 10^6 spores. Molds and actinomycetes in soil were particularly resistant up to 69 C in vacuum. Actinomycetes were the only soil organisms recovered so far at 120 C.

82. THE EFFECT OF SOME DIVALENT IONS ON APPARENT SURVIVAL OF HEAT-TREATED COLIFORM BACTERIA

Abdul-Nuor, B., Nelson, F. E.

(Abstracted in Bacteriological Proceedings, p. 5) 1963

Variations in enumeration conditions influence markedly apparent survival of sub-lethally heated non-sporulating bacteria. Many ions have no greater effect on the heated organisms than on the unheated controls. However, Cu^{++} and Co^{++} do have some marked effects. A basal enumeration medium containing 1 g $NH_4H_2PO_4$, 1 g K_2HPO_4, 5 g glucose, 15 g agar-agar and 1000 ml distilled H_2O (pH 6.8), with incubation at 32 C, was used in most studies. Cu^{++} at 1.2×10^{-5} M reduces the apparent survival of the heat-treated bacteria to approx 10 percent of the count obtained without added Cu^{++}, while unheated organisms give the same count whether or not Cu^{++} is added to the enumeration medium. Addition of 1×10^{-6} M Co^{++} increases apparent survival by 5X to 10X, and 1×10^{-7} M Co^{++} doubles the count. The unheated organisms are not affected until Co^{++} reaches 2×10^{-5} M, at which level this ion also becomes inhibitory to the heated organisms. Using commercially "purified" agar-agar in place of usual bacteriological agar-agar increases the optimum Co^{++} concn to 1×10^{-5} M. Co in B_{12} does not have the same effect as does the inorganic ion.

83. ECOLOGY OF THE SYMBIOTIC GROWTH OF A MESOPHILIC BACTERIUM AT THERMOPHILIC TEMPERATURES

Oates, R. P., Beers, T. S., Quinn, L. Y.

Bacteriological Proceedings, p. 44, 1963

As previously described, a mesophile resembling Bacillus megaterium is capable of growing at 65 C in association with a thermophile resembling Bacillus stearothermophilus. The symbionts demonstrate vigorous cellulose digestion which is not evident when either is in pure culture. Bacteria-free filtrates of the symbiotic culture protect the mesophile against thermophilic temperatures, which are otherwise lethal to it. The mesophile essentially retains the same biochemical reactions following incubation at 65 C in filtrates of the mixed culture as it shows with serial passage at 25 C. Ecological studies of the pure and mixed cultures of this mesophile and thermophile show complex interrelationships in metabolic patterns. The mesophile has been shown to remove or convert a number of chemical compounds which are present in the basal medium at levels toxic to the thermophile. On the other hand, the thermophile establishes suitable ecological conditions for the continuous growth of the mesophile at 65 C. When both organisms are growing together at thermophilic temperatures, the composite metabolic pattern is markedly

83. Cont'd

different than that of either the mesophile or the thermophile.

84. HEAT STERILIZATION OF CANNED FOODS STUDIED

Food and Drug Packaging, v. 8, no. 12, p. 6, 1963

A solution to the problem of the slow rate of heat transfer in heat sterilization of canned foods is being developed by the Food Preservation Division, Australian Commonwealth Scientific and Industrial Research Organization, Canberra, Australia. A prototype mechanical cooker spins the cans on an inclined belt while they are heated by steam at atmospheric pressure. The rotating cans are also sprayed with cool water to reduce the length of time the food is held at the processing temperature.

85. UPPER TEMPERATURE LIMIT OF LIFE

Kempner, E. S.

Science, v. 142, pp. 1318-1319, 1963

The highest growth temperatures which have been confirmed for microorganisms are 72-75 C. The highest temperature in hot springs of Yellowstone National Park, Wyoming, which contain obvious algal mats is 73 C. Waters above 73 C are perfectly clear. Incorporation of radioactive phosphorus (supplied as $H_3P^{32}O_4$) into the cell nucleic acids was considered a metabolic test for growth of organisms in hot spring waters. Samples with radioactive phosphorus were incubated 48 hours in the pools from which they were collected. Pool runoff and pool temperatures ranged from 57 - 93 C. Nucleic acids were extracted with trichloracetic acid, the samples dried, and radioactivity determinations made with a scaler. No evidence for active growth was observed above 73 C. The author discussed several concepts for the temperature limitation related to destruction of proteins, DNA or RNA. Limitation of amino acid acceptance by soluble RNA was considered a plausible explanation.

86. HEAT RESISTANCE OF CLOSTRIDIUM BOTULINUM TYPE E SPORES

Graikoski, J. T., Kempe, L. L.

Bacteriological Proceedings, p. 3, 1964

C. botulinum Type E spores of seven strains were tested in respect to heat activation and their resistance to heat. Spores of the various strains were produced in 5 percent Trypticase, 0.5 percent Peptone, and 0.2 percent glucose media with incubation at 33 C for 24 to 48 hr. Washed-spore suspensions were diluted with 0.067 M PO_4 buffer (pH 7.0) to a final concentration of 10^7/ml. The spores were heated for various time intervals at temperatures between 55 and 90 ± 0.5 C. Survivors were enumerated in beef infusion agar contained in Miller-Prickett tubes. At temperatures of 65 C or lower, little inactivation of the spores occurred. At 70 and 75 C, significant numbers were inactivated within 20 min. An increase in count occurred upon further heating for 40 to 60 min, indicating activation of dormant spores. At 85 and 90 C, survivors were obtained after heating for 120 and 60 min, respectively. Of the heat survivors of three strains subcultured into TPG media, two produced toxic cultures. Spores produced by the heat survivors were similar in their resistance to the stock suspensions. This variation in heat resistance in the spore suspensions can be explained by the

86. Cont'd

ability of Type E cultures to produce three phases of growth with different physiological characteristics.

87. IONIZING RADIATIONS IN THE PROCESSING OF PLANT AND ANIMAL PRODUCTS

Nickerson, J. T. R., Proctor, B. E., Goldblith, S. A.

Food Technology, v. 10, no. 7, pp. 305-311, 1956

Commercial sterilizing doses for certain vegetables, fishery, and miscellaneous food products were found to be in the range 1.5-2.0 X 10^6 rep cathode rays in experiments performed with a Van de Graaf accelerator rated at 3 million electron volts. The radiation resistance of Clostridium sporogenes suspended in pureed spinach was increased when compared to air-packed samples irradiated at room temperature by irradiating in nitrogen, vacuum, freezing and vacuum, and freezing. Vegetable products inoculated with 160-1,800 C. sporogenes spores per g required the aforementioned dose range to provide no growth in any of ten samples.

88. PHYSICAL AND CHEMICAL FACTORS MODIFYING THE SENSITIVITY OF CELLS TO HIGH-ENERGY AND ULTRAVIOLET RADIATION

Hollaender, A.

Symposium on Radiobiology, pp. 285-295

J. J. Nickson, Editor

John Wiley and Sons, New York, 1952

A review of factors modifying radiation sensitivity is presented. Cells are more sensitive to heat after X-ray as well as ultraviolet irradiation. An Escherichia coli strain, however, can be reactivated several hundred-fold if incubated at 40 C compared with incubation at 30 C after UV exposure. The effect is about five-fold after X-ray treatment. Photoreactivation after exposure to UV irradiation at 2537 Å is limited to the wave length range of 3650-4500 Å. No significant photoreactivation occurs after X-ray exposure. Infrared around 10,000 Å given before X-irradiation will increase the effectiveness of X-radiation in producing mutations in Aspergillus terreus. The infrared alone is without effect in experiments which minimize a rise in temperature. Very dry A. terreus spores display a striking increase in X-ray resistance. Aerobically grown E. coli B/r irradiated in nitrogen and in oxygen give a survival ratio at 60,000 r of $N_2/O_2 \doteq >1000$. Nitrogen can be replaced with helium, hydrogen,

89. A MATHEMATICAL MODEL OF RADIATION AND POPULATION OF CELL COLONIES. I. TWO-DIMENSIONAL RANDOM-WALK MODEL

Bellman, R., Elkind, M., Kotkin, B.

U. S. Government Research Reports, v. 38, no. 22, p. 106, November 20, 1963

RAND Corporation, Santa Monica, California, Memo RM365

NIH Grant RG9608

AD-411 864

To study the effect of radiation on the population of cell colonies, a simple model is constructed that follows a cell through a two-dimensional random walk, where one dimension represents state of growth and the other represents state of health or number of sites damaged. The cell is subjected to radiation exposure at prescribed times and doses which can be varied. The cell will eventually divide or become sterile. An IBM 7090 FORTRAN program of the Monte Carlo procedure presents a statistical summary of the results at the absorbing barrier.

88. Cont'd

or carbon dioxide without significantly changing sensitivity. *E. coli* B/r grown anaerobically and irradiated anaerobically are extremely radioresistant. If the sensitivity of the extreme cases is compared, a factor of 10 is found for cells grown aerobically and irradiated in the presence of oxygen, and those grown anaerobically and irradiated in the absence of oxygen.

21 references

90. LACK OF RADIATION ALSO A PROBLEM

Missiles and Rockets, v. 15, no. 2, p. 27, 1964

While the effects of ionizing radiations are being heavily studied, little has been done on the subject of the complete lack of such radiations. Observations by University of Bern scientists for several years in the Simplon tunnel, which has been proven to be free of any traces of cosmic radiation, show that such effects also exist. Artemia eggs showed only a 60-70 percent rate of offspring. Hordexum seeds became sterile after six months, and algae did not grow after a few weeks.

91. OXYGEN PROTECTION OF BACTERIOPHAGE T1 AGAINST IONIZING RADIATIONS

Bachofer, C. S., Pottinger, M. A.

The Journal of General Physiology, v. 40, no. 2, pp. 289-310, 1956

Bacteriophage T1 was suspended in distilled water and 0.001 M phosphate buffer, saturated with oxygen, nitrogen, hydrogen, and carbon monoxide and irradiated with ultraviolet, gamma rays (up to 37,500 r) and X-rays. In contrast to most biological systems which are sensitized by oxygen to ionizing radiations, oxygen protects T1 bacteriophage against both irradiation and hydrogen peroxide inactivation. The protective effect was greater in distilled water than in buffer. Studies with different gases suggested that phage is sensitive to OH radicals. This was confirmed in experiments which showed that the combined effect of hydrogen peroxide and UV acting simultaneously was greater than the effect due to these agents acting separately. It is proposed that the protective effect of oxygen is due to a reaction between the phage and oxygen, and this complex confers stability upon the phage.

17 references

92. SYNERGISTIC ACTION OF ETHYLENEDIAMINETETRAACETATE AND RADIATION ON YEAST

Bair, W. J., Hungate, F. P.

Science, v. 127, no. 3302, p. 813, 1958

This work was directed at determining whether ethylenediaminetetraacetate (EDTA) would decrease or prevent plutonium uptake in growing cells and permit the use of plutonium as an alpha source in microbial cultures. The growth response of a diploid strain of Saccharomyces cerevisiae toward plutonium and EDTA was recorded in an autoturbidimeter. Growth was not altered by EDTA (usually 3×10^{-4} M) but was delayed approximately 3 hours by plutonium (0.5 μc/ml). A synergistic effect in growth inhibition was observed when both materials were present. Appropriate experiments indicated that less plutonium was in the cells when EDTA was present than in its absence. The EDTA effect is therefore not due to an increased radiation dose in cells exposed to EDTA and plutonium. Similar results were obtained in experiments with EDTA and tritium (90 mc/ml). EDTA probably increases the apparent radiosensitivity of yeast by inducing a general change in the electrolyte balance of the cell.

93. AN OBSERVED 'OXYGEN EFFECT' DURING GAMMA-IRRADIATION OF DRIED BACTERIAL SPORES

Tallentire, A.

Nature, v. 182, pp. 1024-1025, 1958

The influence of air on the radiosensitivity of Bacillus subtilis spores was investigated. An aqueous suspension of spores and kaolin was spray-dried to yield a primary dried powder. Samples of this powder were further dried for 6 hr at 0.001 mm Hg to obtain a secondary dried powder. Portions of this last powder were sealed at 0.001 mm Hg and also under dry air. These preparations and primary dried powder sealed in air were irradiated with gamma rays over the range 0-325 krad, and dose vs log percent survivor curves obtained. The presence of air during irradiation (oxygen effect) greatly increases the kill of spores in secondary dried powder. At 100 krad, for example, the log percent survivors in secondary dried powder under reduced pressure is approximately 1.5 but is -0.3 in air. Spores in secondary dried powder irradiated in air are more sensitive to gamma radiation than similarly irradiated spores in primary dried powder. The observed inverse relationship between moisture content and radiosensitivity is contrary to what would be expected from a consideration of the more usually reported protective effect resulting from cell dehydration.

94. THE ELECTROPHORETIC SEPARATION OF A CULTURE OF ENTEROCOCCI INTO RADIATION RESISTANT AND NON-RESISTANT CELLS

Sadoff, H. L., Green, J. H.

Bacteriological Proceedings, p. 20, 1959

A strain of enterococcus was isolated from a canned meat product which had been subjected to 2 X 10^6 rep of gamma radiation. Subsequent investigations showed that approximately one percent of the cells of this strain are radio resistant. Routine methods of sub-culturing were unsuccessful in separating resistant and non-resistant fractions, even when survivors from intensive radiation were cultured. The 90 percent killing dose, D_{90}, for the resistant organisms is of the order of 2 X 10^5 rep which is six to eight times the magnitude of that for the susceptible cells. In order to be able to conduct chemical studies of the mechanism of radio resistance in this organism, it was necessary to devise a method for separating the resistant bacteria from the total population. Since the resistant cells appear to constitute a constant fraction of the culture, a physical method of separation was attempted. A 30-fold concentration of resistant cells was accomplished by the use of gradient electrophoresis. The technique consists of suspending washed cells in 20 percent glycerol and establishing a

94. Cont'd

potential and pH gradient across this medium. Under the conditions employed experimentally, buffers at pH 4 and pH 6.5 and potential of 150 volts, the radiation resistant cells migrate toward the cathode and the susceptible cells toward the anode. Radiation resistance in vegetative bacteria is known to exist and the phenomenon may be wide spread in nature.

95. SOME EFFECTS OF HEAT AND OF IONIZING RADIATION ON GERMINATION AND SUBSEQUENT DEVELOPMENT OF SPORES OF BACILLUS MEGATERIUM

Levinson, H. S., Hyatt, M. T.

Bacteriological Proceedings, pp. 40-41, 1959

Studies of the effect of ionizing radiation on bacterial spores have, generally, not distinguished between failure of spores to germinate and failure of the germinated spores to grow and to form colonies. Bacillus megaterium spores, irradiated (0.8 megarads) under the beam of the Van de Graaff accelerator retained their capacity to germinate. In a phosphate-buffered medium containing glucose and sulfate, irradiated spores become stainable and consume oxygen at a rate equal to that of unirradiated spores. After 110 to 130 min of incubation, the slope of the oxygen consumption rate curve for unirradiated spores again increases coincident with elongation, but irradiated spores continue to consume oxygen without a rate increase. While 75 to 80 percent of the germinated unirradiated spores divide, irradiated spores do not go on to cell division. Since irradiated spores do not divide in any medium we have thus far investigated, we are uncertain as to their viability. This viability problem becomes increasingly difficult to resolve, since at high doses e.g.,

95. Cont'd

1.8 megarads, many of the irradiated spores become stainable, even in the absence of glucose. However, even after 1.8 megarads of irradiation, there is but little decrease in the rate of oxygen consumption during the period corresponding to germination. Spores heated at temperatures from 86 to 90 C for 15 min, also appear to have retained the ability to germinate, but few of the germinated spores divide. Both heat and irradiation may destroy similar systems responsible for the loss of the capacity of the cell to divide. Differences in ultraviolet absorption spectra of extracts of irradiated, unirradiated, and heated spores may lend some support to this view. The implications of these results as they concern food preservation are discussed.

96. DETERMINATION OF RELATIVE RESISTANCE OF SELECTED STRAINS OF CLOSTRIDIUM BOTULINUM TO IONIZING RADIATIONS

Townsend, C. T.

National Canners Association, Berkeley, California

Final Report No. 5 for May 14, 1958 - December 13, 1959

1959, 18 pp.

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Spore suspensions of eighteen strains of Clostridium botulinum including serotypes A,B and E were subjected to three or more sublethal doses of ionizing radiation in beef broth, neutral M/15 phosphate buffer, and pork pea broth. The radiation resistance of the strains was compared on the basis of the median doses necessary to destroy 999,900 of one million viable spores (99.99 percent). The following relationships are indicated: (1) the rate of population reduction with increasing amounts of irradiation is essentially logarithmic; (2) most of the more radiation-resistant strains are in the type A group, but there is overlapping between all three types studied; and (3) relative radiation resistance is not constant from one substrate to another.

97. INFLUENCE OF GAMMA RADIATION ON THE MICROFLORA OF CUCUMBER FRUIT AND BLOSSOMS

Etchells, J. L., Costilow, R. N., Bell, T. A., Rutherford, H. A.

Bacteriological Proceedings, p. 47, 1960

During the 1959 harvest season 8 samplings of cucumber fruit and 2 of cucumber blossoms were irradiated in a Co-60 gamma source with a dose rate of 200,000 reps per hr. Each set of samples consisted of 10 to 12 individual lots sealed in plastic bags to provide for dosages from 0 to 3 million reps. Bacteriological test were made on each sample for 8 microbial groups: total aerobes, total anaerobes, aerobic spores, anaerobic spores, coliform bacteria, acid-forming bacteria, yeasts and molds. Pectinolytic and cellulolytic enzyme tests were made on all samples; firmness tests were run on the cucumber samples. Complete sterilization of small-sized cucumbers (3/4 - 7/8" diam) required about 3 million reps. Total microbial populations of cucumber flowers were reduced from about 20 billion organisms per g to less than 100 by this dosage; for cucumber fruit, the reduction was from 60 million per g to 0. Dosages sufficient to elimate the microbial populations from samples of cucumber flowers did not affect the pectinolytic and cellulolytic enzyme

97. Cont'd

activity of such samples. The most resistant organisms encountered were the aerobic and anaerobic spore-forms; usually 1 to 10 spores survived 1.0 to 2.0 million reps. The coliform bacteria, lactic acid bacteria, molds and yeasts were usually reduced to extremely low numbers by 500 to 750 thousand reps. Of these four microbial groups, the coliforms appeared to be the most sensitive to the gamma rays. Increasing gamma dosages resulted in increasing losses in cucumber firmness.

98. THE RADIO RESISTANCE OF BACTERIAL SPORES AT ELEVATED TEMPERATURES

Bellamy, W. D., Erickson, S. T., Lynch, M. E.

Radiation Research, v. 14, no. 4, p. 450, 1961

Suspensions of Bacillus pumilis 6C and B. megaterium ATCC 8245 spores were irradiated over the temperature range -178 C to 110 C with 1.5 MeV electrons. Using glass capillaries and dose rates up to 33 Kr per sec it was possible to limit temperature excursion to 5 min or less. The effects of pre- and post-irradiation as well as simultaneous heating, oxygen during and after irradiation, suspension media, dose rate and duration of heating were examined. The survival curves of B. pumilis spores was first order under all conditions, while curves of B. megaterium varied between first order and higher order. The resistance of anaerobically irradiated B. pumilis spores was nearly independent of temperature between -178 C and +72 C (D_o= 95-105 Kr), while from 72 to 100 C the resistance decreased linearly (D_o= 23 Kr at 95 C). B. megaterium was least resistant at 0 C over the same irradiation temperature range. Both organisms were virtually unaffected by 5 min heating up to 95 C. Aerobically irradiated B. megaterium does not exhibit a minimum sensitivity over the irradiation range -70 to +80 C but is independent of temperature.

98. Cont'd

Dried spores are much more resistant to radiation than spore suspensions both aerobically and anaerobically at elevated temperatures.

99. WATER, GLYCEROL AND OXYGEN AS FACTORS IN RADIATION SENSITIVITY OF BACTERIAL SPORES

Webb, R. B., Powers, E. L.

Radiation Research, v. 14, no. 4, pp. 515-516, 1961

The relationship between radiation sensitivity and water content with and without oxygen was investigated with Bacillus megaterium spores exposed to 1.5 mm Al HVL X-rays. Spores rehydrated on membrane filters are about 8 percent more resistant than in the air-dry state. Spores in water suspension that have never been dried are about 40 percent more resistant than air-dry spores. An additional reduction in radiation sensitivity of about 2.2 is observed for spores in 8.9 M glycerol solution. The oxygen effect in spores suspended in water is 2.0. In 8.9 M glycerol, the oxygen effect is reduced to about 1.3. In air-dry spores, the latent oxygen effect is 1.90 and the immediate oxygen effect is 1.25. These similarities suggest common mechanisms of action in the wet and dry spores. Desiccation and glycerol addition give opposite results. It is evident that glycerol does not protect these spores by the removal of free water as has been suggested for vegetative systems.

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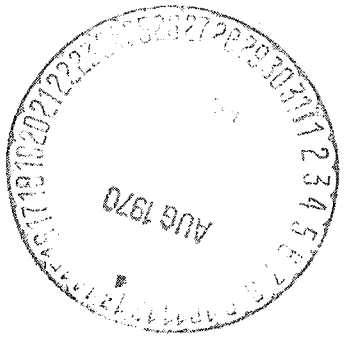

FEASIBILITY STUDY FOR COMBINED METHOD OF STERILIZATION

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VOLUME 2

ENTRIES 100 - 187 AND INDEXES

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RESEARCH LABORATORIES
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100. EFFICIENCY OF INACTIVATION OF DRY T-1 BACTERIOPHAGE BY PROTONS, DEUTERONS, AND HELIUM IONS FROM A 60-INCH CYCLOTRON

Fluke, D. J., Forro, F., Jr.

Radiation Research, v. 13, pp. 305-317, 1960

Lysates of Escherichia coli B and T-1 bacteriophage were dried on glass cover slips in an evacuated desiccator. In addition to cyclotron studies, samples were exposed to gamma rays from a Co-60 source. The gamma dose for 37 percent survival of the dry phage irradiated in air is over 4×10^5 rad, while for protons, deuterons and helium ions the same inactivation required 5.25×10^5 rad. About 85,000 rad are required for 37 percent survival of T-1 phage with X-rays in protected suspension. Extreme dryness inherent in the high vacuum condition in the cyclotron may contribute to the high resistance of T-1.

101. ADAPTATION OF MICROORGANISMS TO RADIATION

Shefner, A. M.

Developments in Industrial Microbiology, v. 1, pp. 21-25, New York, Plenum Press, 1960

A review of radiation resistance studies from several laboratories is presented. Several strains of Escherichia coli have been subjected to radiation and it was demonstrated that E. coli strain B is relatively radiosensitive. A radiation resistant mutant of E. coli B was isolated by Witkin in 1946. The mutant strain, B/r, was distinguished from E. coli B by its difference in response to ultraviolet radiation. Witkin demonstrated that while ultraviolet radiation was a selective agent for the more resistant B/r forms which occurred as spontaneous mutations of E. coli B, the radiation also acted as an inducing agent. The number of radioresistant mutants increased in frequency as the dosage of ultraviolet radiation was increased. Gaden and Henley (1953) irradiated E. coli B with Co-60 at 80,000 r/hr for up to 3 hours daily. Samples of the irradiated cultures were transferred to fresh broth, incubated for 17 hours and again irradiated. It was found that the increase in radiation resistance was quite marked as the number of irradiations increased. After 17 irradiations a strain was derived from strain B which was even more radioresistant than strain B/r.

101. Cont'd

Bryson (1949) and Clark (1952) indicate that E. coli B/r had an increased resistance to hydrogen peroxide in the culture medium. In the author's laboratory, E. coli B cells grown in nutrient broth at 37 C were washed and resuspended in M/15 phosphate buffer, pH 7.0 and irradiated with Co-60 at 26,000 r/hr for 4 hours. Portions of the irradiated cells and non-irradiated controls were then plated and incubated. The remaining irradiated cells were then centrifuged, resuspended, and incubated at 37 C for 48 hours. This procedure was repeated through eleven irradiations. The percent of organisms surviving the irradiation dose increased approximately thirty-fold over the course of the eleven radiations. Nonconclusive experiments were also carried on to test the increased resistance of the mutant strain to hydrogen peroxide. A parallel program of radiation exposures of Serratia marcescens strain 9986 showed a gradual increase in the radioresistance of the culture but provided nonconclusive results. The ability of microorganisms to adapt to environmental factors is significant in space microbiology. If photosynthetic gas exchangers are used, the algae may be subjected to a variety of environmental stresses-radiation, toxic vapors, temperature fluctuations, etc. It may be possible to select strains which are more resistant to hazardous factors and have a low spontaneous mutation rate.

102. NITRIC OXIDE AS A MODIFIER OF RADIATION EFFECTS ON SHIGELLA FLEXNERI

Dale, W. M., Davies, J. V., Russell, C.

International Journal of Radiation Biology, v. 4, pp. 1-13, 1961

A distinction between the actions of nitric oxide and oxygen on irradiated bacteria is described. One millimole nitric oxide has a latent effect when given as a pretreatment gas that is removed from the medium prior to anoxic irradiation of Shigella flexneri. The increased radiosensitivity is believed related to fixation of some nitric oxide by unknown cell constituents during the pretreatment period.

103. A POSTIRRADIATION OXYGEN EFFECT IN BACTERIAL SPORES AND ITS DEPENDENCE ON WATER CONTENT

Tallentire, A., Davies, D. J. G.

Experimental Cell Research, v. 24, no. 1, pp. 148-150, 1961

Experiments are described in which damage, induced in spores by irradiating in oxygen, increases after irradiation. Data is presented which shows that water affects oxygen dependent mechanisms which may result in lethal damage in resting cells. An aqueous mixture of Bacillus subtilis NCTC 3610 spores and kaolin powder was freeze-dried and contained 0.4 percent water. Primary dried powder was further dried in vacuum and samples sealed at 10^{-5} mm Hg, under oxygen or dry air. Following Co-60 irradiation to 50 krad (4 to 1 krad/min) samples were stored at room temperature and plated at intervals during postirradiation storage for 72 hr. Viable secondary dried spores decreased rapidly during the first 24 hr and slower during the remainder of the storage period. In contrast, the survival level of spores in secondary dried powder irradiated and stored under reduced pressure does not decrease with storage. Water added to secondary dried spores, which are then irradiated and stored in oxygen, prevents the drop in survival which occurs in the very dry state. Constant survival also occurred in irradiated aqueous spore suspensions,

103. Cont'd

spores in primary dried powder, and in rehydrated secondary dried powder. The increase in radiation-induced damage in secondary dried spores stored in oxygen compared with none following irradiation and storage under reduced pressure indicates that the postirradiation effect involves oxygen dependent reactions in the inactive dry spore. Very small quantities of water are involved. The oxygen enhancement ratio increases with postirradiation storage from 4:1 to 10:1.

104. THERMAL DEPENDENCE OF THE POSTIRRADIATION OXYGEN EFFECT IN DRIED BACTERIAL SPORES

Tallentire, A., Davies, D. J. G.

Abstracts of Papers, Second International Congress of Radiation Research, Harrogate, England, August 5-11, 1962

Spores of Bacillus subtilis distributed on an inert inorganic solid substrate were dried by pumping under reduced pressure (10^{-5} mm Hg) for six hours and then exposed in oxygen at room temperature to Co-60 gamma rays. Immediately after irradiation the spores were stored in oxygen at temperatures ranging from -196 to 50 C for periods up to 72 hr. At 37, 45, and 50 C the probability of lethal damage from post-irradiation oxygen-dependent mechanisms increases with storage to a maximum which is about 2.6 times greater than that immediately after irradiation. The rate at which this maximum is attained increases with temperature of storage. Post-irradiation storage at 0, 10 and 25 C for 72 hr does not result in maximum probability of damage, but dependence of rate of increase with temperature is observed. At -196 C lethal damage from oxygen-dependent mechanisms is unchanged during postirradiation storage. The kinetics of this post-irradiation oxygen effect have been examined with a view to obtaining a value for the apparent activation energy.

105. STUDIES ON THE POSTIRRADIATION OXYGEN EFFECT IN BACTERIAL SPORES

Tallentire, E., Dickinson, N. A.

The Journal of Pharmacy and Pharmacology Supplement, v. 14, pp. 127T-128T, 1962

In the application of radiation sterilization, the lethal efficiency of radiation can depend on the storage conditions of the irradiated material. Preliminary experiments are described aimed at determining the mechanisms of these postirradiation effects. Samples of kaolin powder contaminated with Bacillus subtilis spores were dried at less than 10^{-5} mm Hg, sealed under vacuum and treated at 22 C with various doses of gamma radiation from a Co-60 source. Samples were then stored in controlled gaseous atmospheres at 25 C. Exponential dose/survival curves were plotted, and the slopes estimated using the expression: surviving fraction = e^{-kD}, where k is the slope and D the dose in Krad. Higher values of k indicate greater lethal efficiency. The greatest lethal efficiency results from postirradiation storage of spores in oxygen for 48 hours ($k = 0.045$ Krad^{-1}). The lowest efficiency occurs for identical oxygen treatment preceded by exposure to nitric oxide for 15 min ($k = 0.010$ Krad^{-1}). Related experiments

105. Cont'd

indicate that development of the postirradiation oxygen effect can be arrested by removing the oxygen, and can be restarted by re-admitting oxygen to the dried spore system. Treatment with nitric oxide between exposures to oxygen, however, prevents further development of the oxygen effect. The authors concur with the views of Powers, Webb and Kaleta (Proc. Natl. Acad. Sci., v. 46, pp. 984-993, 1960) on the scavenging role of nitric oxide, and infer that the post-irradiation oxygen effect results from an association of free radicals with oxygen. Potentially harmful radicals can be removed even after exposure to oxygen, and after partial development of the oxygen effect. Since these radicals are also harmless at reduced oxygen pressure, the authors propose that gaseous oxygen maintains an unstable oxygen-radical complex which is responsible for the postirradiation oxygen-dependent lethal effect.

106. INACTIVATION RATE STUDIES ON RADIATION RESISTANT NONSPORING SPOILAGE BACTERIA

Duggan, D. E., Anderson, A. W., Elliker, P. R.

Bacteriological Proceedings, p. 22, 1962

Factors influencing the resistance of nonsporing spoilage bacteria found to survive high doses of radiation in food were studied. Raw pureed meat substrates were developed that could be pipetted for quantitative bacteriological studies. Gamma radiation survival curves were determined in raw beef using four strains of Micrococcus radiodurans and one culture of Brevibacterium oregonium. B. oregonium was as resistant as R_1, the most resistant strain of M. radiodurans. Survival of the R_1 strain was significantly greater in raw beef and raw chicken than in raw fish or in cooked beef. Growth of the cells in broth or in beef did not affect radiation resistance in beef. Initial cell concentration appeared to have no effect. The most resistant cultures were reduced by a factor of about 10^{-5} by 3.0 Megarad, and by a factor of over 10^{-9} by 4.0 Megarad. Freezing did offer significant protection to R_1. No differences in resistance were observed when the cells were irradiated at 0 C and at 20 C. However cells irradiated at 40 C and at 50 C were significantly more susceptible to radiation kill. Preirradiation heat treat-

106. Cont'd

ment lowered the radiation resistance. Rate of inactivation did not appear to differ when cells were exposed in buffer at pH 5, 7 or 9. Thioglycollate or cysteine in buffer did not seem to protect cells but ascorbate enhanced radiation inactivation.

107. THE EFFECT OF GAMMA RADIATION ON NITROGEN TRANSFORMATIONS IN SOIL

Vela, G. R., Wyss, O.

Bacteriological Proceedings, p. 24, 1962

A study has been made to determine which of the various processes involved in the nitrogen cycle in the soil will become inoperative at measured levels of gamma radiations. The radiation doses at which the different nitrogen transformations were affected varied widely; nitrogen fixation was permanently inhibited by 0.25×10^6r while urease activity was stimulated by doses of over 8 times that value. The mechanisms by which these sensitivities manifest themselves are also quite different one from the other. Thus, using the soil as a biochemical entity, we have observed that its nitrogen-fixing ability is a discontinuous function of the radiation dose as if it were dependent upon the survival of a single microorganism capable of fixing atmospheric nitrogen. On the other hand, nitrification gives a continuous response to the radiation dose; the nitrifying ability of soil subjected to increasing increments of gamma rays approaches zero asymptotically. The release of ammonia from urea by these soils is enhanced by doses of radiation up to two million roentgens per gram of soil; apparently

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ammonification is carried out by the surviving spore-formers.

108. COMPARATIVE RESISTANCES OF STRAINS OF CLOSTRIDIUM BOTULINUM TYPES A AND B AND P. A. 3679 TO GAMMA RAYS

Anellis, A., Koch, R. B.

Bacteriological Proceedings, p. 30, 1962

One hundred two strains of C. botulinum (56 strains of Type A, 43 Type B, and three non-toxigenic strains which could not be typed), were examined for resistance to gamma rays. When these organisms were suspended in neutral phosphate buffer in concentrations of 10^4 spores per tube, the threshold sterilizing dose appeared to be 1.4 Mrad. Partial survival to 1.4 Mrad was shown by 10.7 percent Type A strains, 18.6 percent Type B strains, and one of three non-toxigenic strains. Overall, Type A strains indicated higher radioresistances than Type B strains, although there was overlapping. Representatives of the most resistant strains had D values of 0.317 to 0.336 Mrad; the D values of an intermediate group were 0.224 to 0.253 Mrad, and the most sensitive strain studied, 51B, had a D value of 0.129 Mrad. The radioresistance of P. A. 3679, strain S_2, was comparable to the intermediate C. botulinum group (D=0.209).

109. HEAT AND GAMMA-RADIATION RESISTANCE OF

BACILLUS MEGATERIUM SPORES

Tallentire, A., Chiori, C. O.

Journal of Pharmacy and Pharmacology Supplement,

v. 15, pp. 148T-149T, 1963

This report is part of an investigation in which the heat resistance of bacterial spores is compared with resistance to other physical agents including ionizing and ultraviolet radiations and dehydration. Bacillus megaterium spores were produced in a chemically defined liquid medium containing Mg and Fe as sole divalent metallic ions and glucose, L-glutamic acid and L-asparagine as carbon and nitrogen sources. Washed spore suspensions were heated for different periods in a bath at 100 C. Exponential time/survival curves were plotted and the slopes used as a measure of heat resistance. Similar plots were prepared following exposure of aerated spores to Co-60 gamma rays at 22 C. Mg and Fe or Mn were essential for sporulation. Spores produced in the medium containing Mg and Fe are the least heat resistant; substitution of Fe by Mn plus Ca doubles heat resistance; Addition of Ca to the Mg-Fe medium does not affect heat resistance; addition of Mn to the Mg-Fe medium

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gives spores of intermediate heat resistance as does addition of both Mn and Ca. Gamma radiation resistance was not related to divalent metallic ion content of the medium.

110. A DEPENDANCE ON WATER CONTENT OF BACTERICIDAL EFFICIENCY OF GAMMA-RADIATION

Tallentire, A., Dickinson, N. A., Collett, J. H.

The Journal of Pharmacy and Pharmacology Supplement, v. 15, pp. 180T- 181T, 1963

Bacillus megaterium spores dried on kaolin were equilibrated in vacuum to known water vapor pressures in the range 5×10^{-4} to 21 torr. Gamma irradiation (Co-60) of spores of different water content was carried out at 22 C in the absence of oxygen. To avoid lethal damage from the post-irradiation oxygen effect, samples were then soaked with water in anoxia before exposure to the atmosphere. The lethal efficiency of radiation increases by about 35 percent in changing from the driest spore state to one in which they are at 100 percent relative humidity at room temperature (23 C). Radiation efficiency increases over the vapor pressure range 4.6 to 8.0 torr, is unaffected by a 10,000-fold decrease in water vapor pressure below 4.6 torr; and above 8.0 torr, when efficiency is highest, remains unchanged over a threefold increase in equilibrium pressure. High efficiency is also observed when spores on kaolin are irradiated in the wet state. Part of the lethal damage induced in spores by gamma radiation can thus be mediated by water. The authors stress that consideration

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should be given to all factors which affect bactericidal efficiency of a given radiation dose from the viewpoint of radiation sterilization, since water vapor partial pressures may be on either side of the critical range.

111. THERMAL ANNEALMENT AND NITRIC OXIDE EFFECTS ON FREE RADICALS IN X-IRRADIATED CELLS

Ehret, C. F., Smaller, B., Powers, E. L., Webb, R. B.

Science, v. 132, pp. 1768-1769, 1960

Studies on the influence of physical and chemical factors on the response of dry spores to X-irradiation, as measured by colony-forming capacity, have revealed a systematic sensitivity to such factors as gas and temperature during and after irradiation. This report presents evidence for the existence of long-lived free radicals which are postulated to form lethal complexes with oxygen, or rendered harmless if scavenged by chemical recombination or annealed by thermal energy. Free radical spectra were obtained by electron spin resonance spectroscopy after irradiation at doses of 4000 kr; weaker signals were obtained at doses as low as 250 kr. No signals were found with unirradiated spores. In viability studies, exposure of spores of Bacillus megaterium to higher temperatures after anoxic irradiation results in thermorestoration of a considerable fraction. If anaerobically irradiated spores are exposed to oxygen before annealment, no part of the potential damage is reversible. Nitric oxide enhances survival of anaerobically irradiated spores. Data was obtained in support of the hypothesis that the protective action of nitric

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oxide could be explained by its scavenging action on long-lived oxygen-reacting radicals.

112. STUDIES ON THE DOSE REQUIREMENT FOR THE RADIATION STERILIZATION OF MEDICAL EQUIPMENT: I. INFLUENCE OF SUSPENDING MEDIA

Burt, M. M., Ley, F. J.

Journal of Applied Bacteriology, v. 26, no.3, pp. 484-489, 1963

Material containing Bacillus pumilis spores, and liquid suspensions of these spores received 2.5 megarad gamma radiation. Neither the kind of medium or supporting surface affected radiation resistance except under local anoxic conditions.

113. STUDIES ON THE DOSE REQUIREMENT FOR THE RADIATION STERILIZATION OF MEDICAL EQUIPMENT: II. A COMPARISON BETWEEN CONTINUOUS AND FRACTIONATED DOSES

Burt, M. M., Ley, F. J.

Journal of Applied Bacteriology, v. 26, no. 3, pp. 490-492, 1963

The interruption of dose delivery, as might occur in plant operation, would not affect the sterilizing efficiency of gamma radiation.

114. THE INFLUENCE OF SUBSTRATE ON THE RADIATION RESISTANCE OF CLOSTRIDIUM BOTULINUM

Grecz, N., Walker, A. A., Anellis, A.

(Abstracted in Bacteriological Proceedings, p. 4) 1963

Survivors of C. botulinum 33A after 0.7 and 0.9 Mrad of Co-60 were determined in beef dinner, borate buffer and tris buffer (all at pH 6). The temperature during irradiation was controlled at -196, -150, -100, -50, -20, 0, +20, +40, +60, +80 and +90 C. The radiation survival curves formed a plateau which was unaffected by temperature variations at the region below -50 C (beef dinner) and -100 C (borate and tris buffers) at both 0.7 and 0.9 Mrad.

The lowest radiation resistance of spores represented a shallow trough in the survival curves at the following temperatures.

	0.7 Mrad	0.9 Mrad
Beef dinner	0 C	+40 C
Borate buffer	-20 C	0 C
Tris buffer	+20 C	-

Above the most sensitive area, the radiation resistance of spores steadily increased to form a sharp peak which varied according to substrate and amount of radiation received and was as follows.

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	0.7 Mrad	0.9 Mrad
Beef dinner	> +90 C	+85 C
Borate buffer	+75 C	+65 C
Tris buffer	+65 C	-

The survival curves in borate and tris buffers coincided at temperatures below 0 C. The curves separated above 0 C showing a markedly higher survival in borate buffer.

115. EFFECT OF LIQUID NITROGEN TEMPERATURE ON RADIATION RESISTANCE OF SPORES OF CLOSTRIDIUM BOTULINUM

Grecz, N., Snyder, O. P., Walker, A. A., Schneider, M. D., Anellis, A.

Bacteriological Proceedings, p. 4, 1963

An apparatus consisting of a Dewar flask and a relay system controlling the flow of liquid nitrogen permitted the irradiation of samples in tin cans or pyrex tubes at any temperature ranging from 0 C to -196 C with an accuracy of ± 1.5 C.

An inoculated pack comprising 320 cans of ground beef containing 5×10^4 spores per can of C. botulinum 33A was irradiated with Co-60 at -196 C and 0 C. Incubation was carried out at 30 C for 6 months. Approximately 0.9 megarad more radiation was required to inactivate the spores at -196 C than at 0 C. Cans treated at -196 C showed partial spoilage at 3.6 megarad and no spoilage at 3.9 megarad, the corresponding spoilage - no spoilage doses at 0 C were 2.7 and 3.0, respectively. The majority of positive cans swelled in 2 to 14 days; occasional swelling occurred as late as 33 days. At 1.0 - 1.5 megarad swelling of cans was more rapid than in unirradiated controls, whereas at progressively higher doses

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swelling was delayed proportionally to the radiation dose received. The remaining non-swollen cans had no toxin after 6 months of storage, although occasional cans contained dormant toxigenic spores.

The radiation resistance of C. botulinum 33A in phosphate buffer also increased somewhat at -196 C as compared with 0 C, although the increase was not as marked as in ground beef. The D-value was 0.322 megarad at -196 C and 0.304 megarad at 0 C.

116. MICROBIAL SURVIVAL IN GAMMA-IRRADIATED SOILS OF DIFFERING PHYSICAL-CHEMICAL COMPOSITION

Hervey, R. J., Williams, Z. M., Krise, G. M.

Bacteriological Proceedings, p. 4, 1963

Autoclaved soils employed for culturing the fungus, Phymatotrichum omnivorum, displayed low-level toxicity. Gamma irradiation was explored as an alternative to heat sterilization. Air-dry soils, of widely differing physical-chemical composition (Pullman clay loam -- cl 34 percent, sand 36 percent, org mat 1.4 percent and Houston Black clay -- cl 58 percent, s 6 percent, om 2.8 percent), were irradiated with 874 and 1,747 thousand rads of Gamma rays (from 2,000 curie Co-60 source). One-hundred g soil and 10 g sorghum-seed samples in glass containers were exposed. Microbial survival was determined on 4 liquid and agar media; total and heat resistant (80 C -10 min) counts on tryp -glu soil ext and nutrient agar dilution plates. Low irradiation killed all soil fungi, seed embryos and seed-borne organisms. Neither dosage killed all soil bacteria and actinomycetes (99.64 - 99.71 percent under low; 99.998 percent under high). Clay and organic matter had no protective effect. Percentage-wise, more microbes survived low irradiation in sandy, low organic PCL than in clayey, high organic HBC; high irradiation

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survival (both soils) was approximately equal. Higher percentages of all heat-resistant organisms (composing 1.4 and 5.7 percent of total populations) of PCL (containing 15.3 mill/g) and HBC (9 mill/g) survived irradiation than heat-susceptible organisms. Heat and irradiation survival percentages in all actinomycetes (composing 18 - 19 percent of tot pop) were generally greater than in bacteria and heat-resistant actinomycete survival (both dosages) greater than bacterial spores. Mainly aerobes, preferring complex organic media, survived; a few utilized NH_4NO_3 N.

117. INACTIVATION OF YOUNG ENCYSTING AZOTOBACTER CELLS BY IONIZING RADIATION

Roberts, T. L.

Bacteriological Proceedings, p. 4, 1963

Young encysting cells of Azotobacter strains appear to be very sensitive to radiation as evidenced by their rapid inactivation by ultraviolet radiation. Increasing resistance of maturing cysts to gamma radiations has been reported (J. Bact. 84:119, 1962). These findings indicate that young encysting cells of Azotobacter vinelandii 12837 might serve as reliable biological sensors, or dosimeters, of ionizing radiations; therefore, inactivation by gamma rays, X-rays, and protons was investigated. Seventy-two-hour cells of A. vinelandii suspended in water were exposed to Co-60 gamma rays, 250 Kvp-18 Ma X-rays, and 14 Mev protons. After irradiation, colony counts were made and percent inactivation determined. Gamma and X-ray doses of 30 to 100 r inactivated 10 to 30 percent of the cells, while about 90 percent of the cells were inactivated by 6,000 r. With protons 20 to 25 percent of the cells were inactivated by 30 to 100 rad while about 75 percent were inactivated by 6,000 rad. Although no suggestion is made for minimum dose effect, these young encysting cells show similar sensitivity to gamma

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rays, X-rays, and protons as to ultraviolet radiation.

118. EFFECTS OF EXPOSING LYSOGENIC BACTERIA TO A PULSE REACTOR

Ichiki, A. T.

Bacteriological Proceedings, p. 50, 1963

Previous studies by Marcovich have indicated that the lysogenic bacterium Escherichia coli K12 (lambda) could be utilized as a system for detecting and measuring the biological effects of X- and gamma rays delivered at relatively low doses. The purpose of this study is to determine the effects of a pulse from a Godiva type reactor on E. coli K12 (lambda). At distances from 50 cm to 2 m from the center of the core, between 150 to 10,000 rads of fast neutrons and 10 to 600 rads of gamma rays were delivered to the lysogenic system. The radiation effects of the neutrons and gamma rays on the K12 (lambda) are described in the following properties: survival and induction of the K12 (lambda), length of the latent period and burst size of the induced bacteria, inactivation of the lambda phage, and survival of the K12. The biological effects of the mixed field were compared, for the investigated properties, with the K12 (lambda) exposed to a 250 KVP X-ray unit. The radiation effects of the mixed field, which is primarily fast neutrons, differed from X-rays in one noticeable property of the lysogenic bacteria; the

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latent period of the reactor exposed K12 (lambda) appeared to be significantly shorter than that observed with 250 KVP X-rays.

119. REQUIREMENTS FOR DEVELOPMENT OF RADIORESISTANCE IN ESCHERICHIA COLI

Stapleton, G. E.

Bacteriological Proceedings, p. 50, 1963

Escherichia coli grown in glucose-amino acid media with inadequate buffering develop a remarkable resistance to inactivation by ionizing and ultraviolet radiations, heat and chemical agents. The time sequence as well as the nutritional requirements for this phenomenon have been studied in some detail. It is clear that two phases are involved separable on the basis of nutritional requirements. Cells removed from glucose-amino acid-containing media during the stationary phase after the pH has reached 5.0 are large cells and contain about twice the amount of the chief macromolecular components as do sensitive cells, but are not resistant. Washed suspensions of these cells will, however, develop maximal resistance in 4 to 6 hr at 37 C in either complex or minimal salts-glucose media if the pH is kept low. It is known that these very conditions alter the enzyme concentrations of this species as well as the end products of glucose metabolism. Two mutant strains have been found that cannot develop this resistance under routine conditions. The nature of the metabolic block in these strains that

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prevents the development of resistance is being investigated. It is of interest that the resistant cells of strain B/r (CSH) can express their resistance only when plated, after irradiation, on media containing certain amino acids. These findings are in accord with the idea that repair processes may be the important determinants of radiosensitivity. However, the ability of cells to develop such capacity depends on their preirradiation cultural history.

120. INACTIVATION OF ULTRADRIED BACTERIAL AND MOLD SPORES BY GAMMA IRRADIATION

Davis, N. S., Silverman, G. J., Keller, W. H.

Bacteriological Proceedings, p. 26, 1963

Spores of Bacillus stearothermophilus, B. subtilis var. niger, B. megaterium, Clostridium sporogenes, and Aspergillus niger were placed in ultrahigh vacuum (10^{-9} to 10^{-10} torr) for 5 days and then irradiated to 100,000 and 200,000 rads in a Co-60 irradiator. Specimens were irradiated in vacuum, in air following vacuum treatment, and as desiccated spores not exposed to vacuum. An apparent oxygen effect was demonstrated for ultradried spores exposed to air immediately prior to irradiation. A reduction of from one-third to one-ninth of the viability of spores irradiated in vacuum occurred with vacuum-treated spores irradiated in air. In contrast to this situation, there were no appreciable differences in recovery of spores irradiated in vacuum and in the desiccated state exposed to air.

121 INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

Gambino, J. J., Lindberg, R. G.

First Quarterly Progress Report, October 1-December 31, 1963, NASA Contract NASw-812, Northrop Corp., Hawthorne, California

NASA CR-55553; NSL-64-29-1

Perognathus longimembris were subjected to 1400 r Co-60 irradiation. Oxygen (100 percent) was administered to one group during the irradiation, another group was splenectomized prior to irradiation, and a third group was forced to maintain its body temperature during the 30-day postirradiation period. Survivors occurred in all groups, suggesting that neither hypoxia mechanisms nor the lowered metabolic rate are responsible for the remarkable radiation resistance of Perognathus.

122. A STUDY OF THE EFFECTS OF COMBINING ULTRASONIC AND IONIZING RADIATION UPON BACTERIAL SPORES

Ney, L. F.

U. S. Government Research Reports, v. 38, no. 22, p. S-20, November 30, 1963

Stanford Research Institute, Menlo Park, California,

Final Report No. 2 for June 7, 1960 - June 6, 1961

Contract DA 19-129-qm-1610

Project No. PBU-3289

PB 163 593 OTS

The program included experiments to determine the effects of insonation and irradiation applied separately and in combination to suspensions of Clostridium botulinum spores. At various frequencies, insonation under cavitation conditions for up to 40 min or longer generally results in higher spore counts than in untreated controls. Extended treatment for 60-80 min has a very noticeable killing effect on the spores. The most generally observed effect of combining insonation with irradiation is an enhancement of the killing effect of the ionizing radiation, even under those conditions which do not produce noticeable killing by insonation alone.

123. THE EFFECT OF ACRYLAMIDE AND ITS HYDROGENATED DERIVATIVE ON IRRADIATED BIOLOGICAL SYSTEMS

Kozlov, Yu. P., Kalabykha, T. N.

In Effects of Some Chemical Agents on Living Matter, Translation from Doklady Akademii Nauk SSSR (Moscow), v. 152, no. 3, pp. 737-743, 1963

OTS: 64-21994

JPRS: 24050

The influence of aqueous solutions of acrylamide and its hydrogenated derivative propionic acid amide on the degree of polymer grafting on irradiated wheat seeds, on the survival rate of irradiated diploid yeast cells, and on the degree of hemolysis of irradiated human erythrocytes was investigated. In all cases introduction of acrylamide led to inhibitions in the free radical states arising from irradiation while the hydrogenated derivative had a completely opposite effect.

124. THE PHYSIOLOGICAL CHANGES PRODUCED IN YEAST BY ULTRA-VIOLET LIGHT AND BY HEAT

Duggar, B. M., Anderson, T. F.

Science, v. 90, p. 358, 1939

Exposure of Saccharomyces cerevisiae cells to 2650 A ultraviolet radiation followed by heat treatment at 50 C is 2-5 times more lethal than the reverse procedure. Pre-irradiation increases the uptake of methylene blue after heating, but does not affect the respiration rate. Ultra-violet doses which prevent colony development do not affect respiration rate.

125. RESISTANCE OF BACTERIAL SPORES TO GAMMA IRRADIATION

Morgan, B. H., Reed, J.

Food Research, v. 19, pp. 357-366, 1954

Spores of thermophilic anaerobe NCA 3814 were more susceptible to heat treatment at 240 F after receiving 250,000, 600,000, and 1,000,000 rep gamma irradiation from a Co-60 source. Bacillus coagulans and B. stearothermophilus were not rendered more sensitive to gamma radiation by prior heat treatment.

126. PILOT THEORETICAL STUDY OF THE EFFECT OF WEIGHTLESSNESS AND DENSELY IONIZING RADIATION ON SINGLE CELLS

Pollard, E. C., Yiesley, W., Barone, T., Weare, J.
Pennsylvania State University, University Park

Final Report, NASA Grant NsG-182-62, 16.pp.
January 14, 1964

The relationship between the behavior of a living cell and the mechanical stress set up by intermolecular forces in response to the distortion produced by gravity was investigated. Theoretical considerations are given to the question of whether the absence of gravity will affect the behavior of a cell of a living system.

127. COMBINED USE OF IRRADIATION, HEAT AND VITAMIN K_5 FOR THE DESTRUCTION OF SALMONELLA

Licciardello, J. J.

Personal Communication, 1964

Vitamin K_5 sensitizes Salmonella to gamma irradiation only when the substrate (buffer) contains no organic matter and only in the absence of oxygen. There is no enhanced effect of temperature on radiosensitization by vitamin K_5 over the temperature range 32 - 120 F. The radiosensitizing effect of vitamin K_5 is less at the higher temperature than at lower temperatures.

128. EFFECT OF TEMPERATURE ON RADIOSENSITIVITY OF *SALMONELLA TYPHIMURIUM*

Licciardello, J. J.

Journal of Food Science, v. 29, no. 4, pp. 469-474, 1964

Radiosensitivity resulting from simultaneous gamma irradiation and heating was compared with the bacterial destruction which occurred when irradiation and heat were applied in succession. Cells were suspended in whole egg magma and liquid egg during treatment, since the simultaneous treatment technique might be potentially useful in killing *Salmonella* in liquid egg prior to freezing or drying. Radiosensitivity in egg yolk increased in a nonlinear fashion as irradiation temperature increased from 32 to 130 F. The lethal effect was greatest at irradiation temperatures above 120 F. In whole egg, the most significant effect of temperature during irradiation occurred at 110 F or greater, the lethal temperature range for unirradiated *S. typhimurium*. The organism was more resistant in egg yolk (pH 6.15-6.25) than in whole egg (pH 7.6-7.8). Bacterial destruction was significantly greater when radiation (20,000-125,000 rads) and heat were applied simultaneously than when they were applied consecutively. Theories proposed to explain the increased radiosensitivity at higher temperatures are discussed.

129. NATURE OF THE RADIATION-RESISTANT "TAIL" IN THE SURVIVAL CURVES OF *CLOSTRIDIUM BOTULINUM*

Anellis, A., Grecz, N., Berkowitz, D.

Bacteriological Proceedings, p. 3, 1964

Some factors affecting the survival of occasional spores of *C. botulinum* 33A at radiation doses beyond the exponential range of the survival curves, i.e., the tail portion has been investigated. Survival of spores was observed up to 9.0 Mrad with erratic skips at doses between 2 and 9 Mrad. Spore crops produced from 9.0-Mrad survivors had no unusual radiation resistance as compared with parent spore crops. The resistance of spores was considerably higher in pork-pea broth than in phosphate buffer (pH 7.0). The materials released into these two suspending media from spores irradiated to 4.5 Mrad appeared not to be responsible for increased resistance such as was manifested by "tail" survivors. Furthermore, dense populations of spores inactivated by 4.5 Mrad did not offer any detectable degree of radiation protection to viable spores of *C. botulinum* 33A

130. RADIATION SURVIVAL OF SPORES OF CLOSTRIDIUM BOTULINUM AT 0 C AND -196 C IN TWO SUSPENDING MENSTRA

Grecz, N.

Bacteriological Proceedings, p. 3, 1964

The survival of spores of C. botulinum 33A irradiated with gamma rays in phosphate buffer (pH 7) and in pork-pea infusion broth at doses ranging from 0 to 3 Mrad in 0.2 -Mrad intervals indicated an appreciable degree of protection of spores by pork-pea broth against the lethal effect of radiation. The radioprotection afforded by pork-pea broth was more pronounced when the temperature during irradiation was controlled at 0 C than at -196 C (the temperature of liquid nitrogen). Low temperature during irradiation (-196 C) provided significant protection to spores suspended in phosphate buffer but not to those in pork-pea broth. In fact, 10^7 spores irradiated in pork-pea broth yielded identical survival curves at 0 and -196 C up to 1.4 Mrad but diverged somewhat at doses higher than 1.4 Mrad with somewhat higher survival figures at -196 C. Hence, it could be concluded that pork-pea broth offered more radiation protection to spores of C. botulinum 33A than did the low temperature (-196 C). The fact that no increase in radiation resistance of spores in pork-pea broth could be achieved by lowering the temperature from 0 to -196 C

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(at doses less than 1.4 Mrad) appeared to suggest that spore inactivation under these conditions was predominantly or exclusively by direct-hit radiation effect, whereas at doses larger than 1.4 Mrad the indirect effect seemed to gain some importance. On the other hand, in phosphate buffer there was a significant amount of indirect radiation effect at both radiation temperatures, 0 and -196 C.

131. RADIATION RESISTANCE AND BALANCED SYNTHESIS OF MACROMOLECULES IN ESCHERICHIA COLI

Stapleton, G. E.

Bacteriological Proceedings, p. 40, 1964

Several reports have emphasized the importance of "unbalanced" DNA synthesis in radioresistance of E. coli. The emphasis on high ratios of DNA/RNA or DNA/protein and enhanced resistance may describe the organism studied rather than the phenomenon, since most of the investigations have used a thymineless mutant or its polyauxotrophic derivative. It is well substantiated that "excess" DNA per cell, without imbalance with reference to other macromolecular components, may result from growth of several strains of E. coli in poorly buffered glucose-amino acid media. This condition also brings about radioresistance. Investigation of the kinetics of development of radioresistance in glucose-containing media indicates that "excess" DNA probably results from dissociation of the cell division process and macromolecular synthesis by an abrupt shift in the pH of the medium. The development of maximal radioresistance follows by several hours the development of excess macromolecular components per cell. Some mutant strains of this species fail to develop resistance, but do show the usual increase in DNA per cell. Moreover, in strains that show remarkable radio-

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resistance, there is a strong dependence on postirradiation nutritional supplementation. Most of the available data leads us to suggest that "excess" DNA may be required for enhanced radioresistance, but its activity is interdependent on other cellular systems that can be physiologically and genetically controlled.

132. RADIATION STERILIZATION OF ORANGE JUICE

Dharkar, S. D.

Indian Journal of Technology, v. 2, no. 1, pp. 24-26, 1964

Spoilage organisms in orange juice are sensitized by gamma irradiation to subsequent heat treatment. The object of this study was to determine the optimum radiation dose and optimum conditions for thermal shock. Gamma irradiation doses ranging from 1×10^5 to 7×10^5 rad or heat treatment alone were not effective in sterilizing juice with a microbial count greater than 10^6 per ml. Sterilization was obtained by 4×10^5 rad followed by 15 min incubation at 50 C. A dose of 8×10^5 rad was necessary to sterilize the juice by radiation alone. Neither oxygen, air or nitrogen influenced the survival of irradiated organisms. It is likely that protective agents in orange juice prevented an oxygen effect. The influence of holding temperature following irradiation on survival of microorganisms is noted in the following table. Juice was maintained at each temperature for 15 min and then plated.

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Radiation dose	Colonies per ml Incubation temperature		
rad X 10^5	50 C	60 C	70 C
1	1.2 X 10^7	95	40
2	1.0 X 10^7	28	26
3	1.0 X 10^7	29	20
4	0	0	0

In another experiment, 10^7 Bacillus cereus spores and 10^7 Saccharomyces cerevisiae cells were incorporated into 100 ml of juice. Tubes of juice were irradiated to 2 X 10^5 rad, then heated to 55 C for 15, 30, and 45 min and plated. The results in the following table show that increasing the incubation time after irradiation decreases the number of microorganisms.

Incubation min	Colonies per ml
15	9 X 10^6
30	1 X 10^4
45	1 X 10^4

The yeast cells are more susceptible to postirradiation heat treatment than the bacterial spores.

133. SENSITIZATION OF MICROORGANISMS TO RADIATION BY PREVIOUS ULTRASONIC TREATMENT

Dharkar, S. D.

Journal of Food Science, v. 29, no. 5, pp. 641-643, 1964

Micrococcus radiodurans (radiation-resistant) and Streptococcus faecalis (relatively radiation-sensitive) were suspended in M/15 phosphate buffer, pH 7.0, at 5 X 10^7 cells per ml. Three-ml portions were sealed in ampoules and exposed to ultrasonic waves in a General Electric ultrasonic generator at 300 kc. In order to separate lethal from sensitizing effects, experiments were performed with low transducer power of 10 watts which did not affect viability in exposures for up to 60 min. The organisms were sonicated for 20 min and exposed to Co-60 gamma radiation at a dose rate of 1.8 X 10^5 rad/hr. Sonicated and nonsonicated irradiated cells were plated, and percent survival vs radiation dose plotted. M. radiodurans suspensions received 1.8 - 10.8 X 10^5 rad while S. faecalis was exposed to 15 - 90 X 10^3 rad. The data was subjected to regression analysis. The D_o value (90 percent mortality) for M. radiodurans decreases from 5.8 X 10^5 rad to 2.9 X 10^5 rad, while that for S. faecalis decreases from 37 X 10^3 to 22 X 10^3 rad. Sterilizing doses calculated for

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M. radiodurans based on 10^6 cells are 5.91 X 10^6 rad for radiation alone and 3.01 X 10^6 rad for the combination sonication-radiation treatment. The marked effect of ultrasonic treatment in enhancing the radiation sensitivity of microorganisms indicates its value in radiation sterilization of liquid foods.

134. THE EFFECT OF HEAT AND RADIATION ON THE VIABILITY AND PATHOGENICITY OF CLOSTRIDIUM TETANI

Shoesmith, J. G.

The Journal of General Microbiology, v. 35, no. 3, pp. vi-vii, 1964

Washed spores of a toxigenic strain of Clostridium tetani were heated at 80 C to inactivate residual toxin and vegetative cells and suspended in isotonic buffer, pH 7.0. Spore viability after different periods of heating at 100 C was determined by colony counts on an agar medium, and pathogenicity was determined by the ability to cause tetanus in mice. The mean LD_{50} dose was 3×10^3 untreated viable spores. Heating was continued until suspensions were sterile. Both viability and pathogenicity were inactivated exponentially, but not at the same rate. The time (D value) for a tenfold decrease in viable counts was 15 min, but 25 min was necessary for a tenfold reduction in pathogenicity. Spore suspensions were also treated with Co-60 gamma radiation and examined in a similar way. A greater difference was observed between the inactivation rates of the two properties than was found for heat. The D value for viability was 2.6×10^5 rad and the D value for pathogenicity was 6.0×10^5 rad. The ability of C. tetani spores to synthesize toxin in vivo is more

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resistant to heat and radiation than the ability to form colonies.

135. NEW ASPECTS OF THE OXYGEN CONCENTRATION EFFECT IN X-RAY INACTIVATION OF BACTERIAL SUSPENSIONS

Hollaender, A., Stapleton, G. E.

Federation Proceedings, v. 12, p. 70, 1953

The effect of oxygen in increasing the X-ray sensitivity of bacterial suspensions is pronounced at concentrations between 1 and 8 percent. Radical formation by X-rays in the aqueous medium fails to explain why the entire oxygen effect takes place within such narrow range of oxygen concentration. Previous experiments suggested that the inactivation coefficient for X-irradiated bacteria was dependent on the cell concentration in the suspension. The effect of bacterial cell concentration at various oxygen concentrations has been reinvestigated. A concentration effect was observed only in those suspensions in equilibrium with air. Removal of small amount of oxygen from suspensions in equilibrium with air would result in large changes in the inactivation coefficient. It appears that the previously suggested bacterial concentration effect may result from removal of oxygen from the suspensions by the bacteria. Experiments in which respiratory inhibitors were used seem to confirm this hypothesis.

136. EFFECT OF MENADIOL DIPHOSPHATE (SYNKAVITE) ON THE SENSITIVITY OF E. COLI AND S. CEREVISIAE TO X-RAYS

Kohn, H. I., Gunter, S. E.

Radiation Research, v. 2, pp. 351-353, 1955

The ability of low concentrations of menadiol diphosphate (MDP) to increase the effect of X-rays on micro-organisms was investigated in view of the notable effects of this compound on irradiated tissue cultures. MDP samples from several sources were used at concentrations ranging from 10^{-3} to 10^{-7} M and were not toxic to the cells during several hours of incubation at 25 C. X-rays generated at 250 kv were applied at a dose rate of 200 to 400 r/min. MDP was without effect at the LD_{50} dose (ca 2000 r) for both organisms. At 10,000 r approximately 5 percent of the 10^4 - 10^6 cells/ml survived. MDP did not affect the yeast, but a small increase in E. coli lethality was noted at 10^{-5} M MDP but not at 10^{-3} M.

137. MODIFICATIONS OF X-RAY EFFECTS FOLLOWING THE ADMINISTRATION OF SOME AMINE COMPOUNDS

Baldini, G., Ferri, L.

Radioterapia, Radiobiologia e Fisica Medica, v. 11, pp. 125-145, 1955

Cysteamine (β-mercaptoethylamine) at 50-200 gamma/ml inhibited the growth of Escherichia coli and Mycobacterium. X-ray irradiated E. coli were not protected in the presence of cysteamine. Pantothenic acid enhanced the radioprotective action of cysteamine in rats.

138. FACTORS INFLUENCING THE PROTECTIVE ACTION OF CYSTEINE AGAINST X-RAYS

Gunter, S. E., Kohn, H. I.

Bacteriological Proceedings, pp. 53-54, 1959

The influence of drug concentration, pH, temperature, and reaction time on the ability of l-cysteine to protect against the lethal action of X-rays was studied with Escherichia coli. A dilute suspension of cells in buffered salt solution was exposed to 250 kv X-rays and/or l-cysteine under various conditions. The effect on viability was determined by macrocolony counts. The process by which l-cysteine protects cells appears to occur in at least two stages, (a) an initial period during which the drug reacts with the cell and its environment and (b) the actual irradiation period, the events of which are modified by the presence of the drug. Development of a resistant state during the reaction period is strongly inhibited by low temperature but once this state has developed at higher temperatures chilling does not change the survival. These two stages may overlap when the interval between l-cysteine addition and irradiation is relatively short. Addition of l-cysteine to cells causes a rapid increase in survival which immediately levels off and at temperatures below 37 C slowly increases with time. At any concen-

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tration, the initial level and the nature of the subsequent rise are a function of temperature and pH. The maximum protection attainable under any given set of conditions is determined by l-cysteine concentration and is greatest at 0.1 and 1.0 M. The protection ratio, (D_{37} with l-cysteine)/(D_{37} without l-cysteine), under optimum conditions varied from 4.2 to 6.0 in contrast to values of 3.0 reported for anoxia.

139. CHANGES IN RADIOSENSITIVITY OF ESCHERICHIA COLI AS RELATED TO ALTERED NUCLEIC ACID CONTENT

Billen, D.

Bacteriological Proceedings, p. 54, 1959

Log phase Escherichia coli strains B/r or 15_{T^-} grown in the presence of chloramphenicol for one hr showed less X-ray killing than untreated cells similarly irradiated. A study of the relative importance of the increased cellular content of deoxyribonucleic and ribonucleic acid (DNA and RNA) in this chloramphenicol-induced radio-resistance was initiated with the thymine requiring strain 15_{T^-}. It was observed that the chloramphenicol induced radioresistance failed to develop in the absence of exogenous thymine. Such cells synthesized and accumulated only RNA in relatively large amounts in the presence of the antibiotic. In the absence of both chloramphenicol and thymine these cells synthesized and accumulated RNA and protein in quantities leading to thymineless death. Such cells were more radiosensitive than similar cells grown in the presence of thymine. Thus the increase in survivors among chloramphenicol treated cells seemed to be correlated with the accumulation of "surplus" DNA. The role of DNA in X-ray death was further substantiated in experiments in which the effect of the addition of competitive concentrations of

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5-bromouracil to growing 15_{T-} cells was studied. In similar experiments other investigators have shown that 5-bromouracil was incorporated into the DNA. Although there was a net increase in DNA content per cell the radiosensitivity of such cells was markedly increased. There appeared to be no correlation between these changes in radiosensitivity and delayed growth of such cells (post-irradiation recovery) following the removal of these substances.

140. SIMILARITIES IN THE EFFECTS OF OXYGEN AND NITRIC OXIDE ON THE RATE OF INACTIVATION OF VEGETATIVE BACTERIA BY X-RAYS

Howard-Flanders, P., Jockey, P.

Radiation Research, v. 13, pp. 466-478, 1960

Suspensions of Shigella sonnei and haploid Saccharomyces cerevisiae were irradiated under controlled gas conditions at 250 kv, 15 ma at 1500 rad per min for X-ray doses up to 60 krad. In the absence of irradiation, nitric oxide (5 percent in nitrogen) did not affect survival of the bacteria during 40 min at 1 and 15 C, but only 70 percent survived exposure to the gas at 30 C. Nitric oxide was found to be equivalent to oxygen, molecule for molecule in influencing the radiosensitivity of Shigella sonnei. Radiosensitivity was doubled by 1.6 μM of either gas. The radiosensitivity of anoxic yeast increased by a factor of 1.5-2 with 30 μM nitric oxide.

141. THE PARTICIPATION OF BOUND WATER IN THE RADIATION RESPONSE OF BACTERIAL SPORES

Tallentire, A., Powers, E.L.

Abstracts of Papers, 9th Annual Meeting, Radiation Research Society, Washington, D.C., May 15-17, 1961, p. 510

Previous work at the Argonne National Laboratory has resulted in the presentation of a "radiation sensitivity profile" describing the radiation response of spores of Bacillus megaterium prepared and irradiated under standard conditions. This profile was obtained by exposing air-dry spores at room temperature to X-rays (50 kvp at about 20 kr/min) in different gases and by post-irradiation treatment with heat and gaseous radical scavengers. These spores undoubtedly contain water in a bound state and may contain some free water. The response of spores subjected to extreme drying conditions and irradiated in oxygen is known to differ from that of air-dry spores. This and other conditions led to an investigation of removal of water on the profile referred to above. Following preparation in the usual manner, spores were further dried under high vacuum in conditions of low aqueous vapor pressure and then irradiated anoxically after introduction of nitrogen through traps suitable for removal of water vapor. Exposure to oxygen immediately after

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irradiation yields an inactivation constant of 0.020 kr^{-1} compared with 0.030 for air-dry spores similarly treated. Furthermore, postirradiation treatment with nitric oxide prevents the development of a part of the damage as it does in air-dry spores (class III damage), whereas post-irradiation heat treatment does not, in contrast to air-dry spores. Controlled anoxic rehydration of spores before irradiation in nitrogen results in spores in which the sensitivity is the same as that of air-dry spores but in which thermal restoration has not been observed. In this system there appears to be at least three critical water contents each capable of affecting in part events occurring early after energy absorption.

142. EFFECT OF PRETREATMENT WITH NITRIC OXIDE AND N-ETHYLMALEIMIDE ON THE LEVEL OF SULPHYDRYL COMPOUNDS IN BACTERIA AND ON THEIR SENSITIVITY TO X-IRRADIATION UNDER ANOXIA

Lynch, J. P., Howard-Flanders, P.

Nature, v. 194, no. 4835, pp. 1247-1249, 1962

Nitric oxide increases the sensitivity of anoxic bacteria, insect, plant and mammalian cells to X-irradiations. Anoxia is protective, however, when sulfhydryl compounds are present during irradiation. Experiments were performed to determine whether reduction in protection afforded to Shigella sonnei following treatment with nitric oxide is due to depletion of the intracellular sulfhydryl compounds. Cell suspensions were exposed to X-rays generated at 250 kv and 15 ma with a dose-rate of about 3,000 rad per min. Anoxia was obtained when required by passing oxygen-free nitrogen through the irradiation vessel. Exposure to 1 mM nitric oxide alone for up to 90 min resulted in 40-50 percent loss in viability in cells grown on nutrient agar. The data in the following table has been extracted from survivor curves and demonstrates that pretreatment with nitric oxide or N-ethylmaleimide (NEM), a sulfhydryl scavenger, renders bacteria more sensitive to anoxic irradiation. The amount of sulfhydryl is drastically reduced by these treatments. Cysteine given prior to

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irradiation reverses much of the nitric oxide pretreatment effect.

Pre-treatment	Atmosphere	X-irradiation Krad	Fraction Surviving
1 mM NO, 60 min	oxygen	10	10^{-3}
1 mM NO, 60 min	nitrogen	10	10^{-2}
1 mM NO, 60 min; 1 mM cysteine	nitrogen	10	10^{-2}
———	nitrogen	10	$10^{-1.5}$
———	nitrogen	20	$10^{-3.5}$
———	oxygen	10	$10^{-3.5}$
0.3 mM NEM	oxygen	10	10^{-4}
0.3 mM NEM	nitrogen	10	10^{-3}
0.3 mM NEM; 2 mM cysteine	nitrogen	10	10^{-2}

143. RADIATION RESISTANCE OF ENZYMES IN SPORULATING BACILLUS SUBTILIS

Newcomb, H. R., Rowley, D. B., Wynn, Jr., J. H., Phillips, A. W.

Bacteriological Proceedings, p. 30, 1962

The marked radiation resistance of bacterial spores may be related to changes in radiation resistance of certain enzymes during sporogenesis. Vegetative cells of B. subtilis (ATCC 6633) were harvested from a chemically defined medium after 17 hr, washed, resuspended in 0.02M K_2HPO_4, and incubated. Within the first 8 hr of endotrophic sporulation, 70 percent of the cell population sporulated. During this period, resistance to X-rays (250 Kvp) of the sporulating culture developed 2-3 hr before heat resistance. The radiation resistance of succinic and malic dehydrogenases, glutamic-pyruvic (GPT) and glutamic-oxalacetic (GOT) transaminases were determined. At 0, 4, 8 and 12 hr, enzyme assays were performed on irradiated and non-irradiated cell-free extracts, prepared by sonication and differential centrifugation to separate the extract from the intact spores. Spore homogenates showed negligible enzyme activity. However in cell-free extracts, maximal activity of malic dehydrogenase, GOT and GPT was observed at 0 hr, and succinic dehydrogenase

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at 4 hr. At these times sporulation was negligible. Between 4 and 8 hr, the maximum rate of sporulation occurred at which time ca 10 percent decrease in dehydrogenases and ca 60 percent in transaminases were observed. At 260 krads cell-free extracts from the above stages of sporulation indicated essentially no increase in radiation resistance of the enzymes studied. Between 0 and 12 hr, the percentage enzyme inactivation by radiation (260 krads) increased gradually. The transaminases indicated greater radiation sensitivity than the dehydrogenases.

144. GENETIC ANALYSIS OF X-RAY RESISTANCE IN ESCHERICHIA COLI

Adler, H. I., Copeland, J. C.

Bacteriological Proceedings, p. 59, 1962

Matings between an X-ray resistant Vhf (very high frequency) donor strain of E. coli and a sensitive recipient were made. Many of the progeny are as sensitive as the original recipient parent, a few are as resistant as the donor parent, and a large group are of intermediate resistance. An analysis of the data suggests that more than one distinct class may be present in the intermediate group. The difference in radiation sensitivity of the parent strains may be ascribed to a group of genes, some of which seem to be located on the bacterial chromosome between the genes controlling histidine and proline synthesis. Comparison of the results from X-ray and ultraviolet inactivation studies suggest that many of the same genes are involved in determining response to both kinds of irradiation.

145. THE INFLUENCE OF INCUBATION TEMPERATURE ON THE VIABILITY OF ESCHERICHIA COLI DAMAGED BY PHENOL, X-RAYS AND RADIO-MIMETIC AGENTS

Harris, N. D., Whitefield, M.

Abstracts of Papers, 41st General Meeting, The Society for General Microbiology, Belfast, September 17-19, 1964

Three strains of Escherichia coli were damaged by treatment with phenol, X-rays and the radio-mimetic drugs dimethyl Myleran and chlorambucil. They were then exposed for 24 hr to temperatures in the range 8-37 C before their viability was finally tested by incubation at 37 C. All the strains showed similar patterns of responses. The exposure temperature resulting in the best viability was 15 C for X-irradiated organisms and 30 C after phenol treatment. Change of temperature had little effect on counts from suspensions treated with radio-mimetic drugs. Incubation of X-irradiated E. coli B/r on media containing up to 5 μg/ml of chloramphenicol resulted in low counts. It is concluded that the beneficial effects of incubation at temperatures below 37 C were not due to retardation of protein synthesis, and that the damage caused by X-rays differed from that caused by radio-mimetic agents.

146. STUDIES ON THE RADIATION RESPONSE OF SPORES OF *CLOSTRIDIUM ROSEUM*

Wooley, B. C., Collier, R. E., Clark, J. B.

Bacteriological Proceedings, p. 36, 1964

This investigation was designed to study the radiation response of *C. roseum* during the processes of sporulation and germination. It was determined that the increase in resistance to both ultraviolet and X-irradiation occurred before the spores exhibited thermostability. This resistance was found to occur almost simultaneously with the accumulation of calcium and the biosynthesis of DPA. Experimental evidence shows that a X-radiation dose of 1.5 mega-roentgens does not inhibit germination as measured by the loss of optical density, loss of DPA, and the increase in stainability. However, a dose of only 350 kilo-roentgens will prevent the outgrowth of about 90 percent of the irradiated spores. It appears that the molecular changes responsible for radiation resistance occur early in sporogenesis and that the germination capacity is more highly protected than the capacity of the germinated spore to undergo outgrowth.

147. ENVIRONMENTAL FACTORS AFFECTING PHOTOPROTECTION AGAINST X-RAY DAMAGE IN STAPHYLOCOCCUS AUREUS

Savage, N. L., Howell, R. D., Clark, J. B.

Bacteriological Proceedings, p. 39, 1964

It was reported earlier that Nocardia corallina could be photoprotected against X-ray inactivation. This observation has since been extended to include several other organisms. In attempts to increase the amount of photoprotection, experiments were performed to determine the effect of certain environmental factors on this phenomenon. Variables studied included incubation temperature, cell age, and culture medium used to grow the organisms. All of these variables were shown to affect the amount of photoprotection which could be obtained. The test organism, S. aureus, was photoprotectable if incubated at 37 C but not if incubated at 29 C. Maximal photoprotection at 37 C was obtained with cells in the logarithmic-growth phase. Cultures of the test organism grown on brain heart infusion agar showed a higher degree of photoprotection than cultures grown on nutrient agar. A maximum of 44 percent protection was found when the organism was transferred at 12-hr intervals on brain heart infusion agar prior to the experiment. The results obtained indicate that the physiological state of the organism is important in the degree of photoprotection which can be demonstrated.

148. EFFECT OF ULTRAVIOLET RADIATION ON MICROORGANISMS AS A PRINCIPAL ENTREMAL FACTOR OF SPACE ENVIRONMENT

Federova, R. I.

In "Life Science and Space Research", v. II, A Session of the Fourth International Space Science Symposium, Warsaw, June 3-12, 1963, pp. 305-310, North-Holland Publishing Company, Amsterdam, 1964

The ultraviolet resistance of microorganisms is considered in connection with the protection against germicidal solar ultraviolet radiation which might be afforded by cosmic dust. The possibility of incidental transportation of living microorganisms from one planet to another is of increasing importance in view of the recent decision to sterilize the surfaces of space ships.

Temperatures close to absolute zero are not germicidal, and some species survive in a vacuum of about 10^{-10} mm Hg for a long time. The outer space vacuum of 10^{-16} mm Hg may also not be lethal. The resistance of spores against penetrating radiation is so high that cosmic radiation is not considered a sterilizing factor. Ultraviolet radiation is considered the main obstacle in preservation of viability.

The amount of UV energy required for 80-100 percent lethality varies from 23 to 440,000 erg/cm^2. Spores of bacteria and fungi are 10-40 times more resistant than vegetative forms. Microorganisms such as Aspergillus niger which produce dark colored

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spores are most resistant, but no organisms withstand about 2 X 10^3 erg/cm^2/sec longer than 3-4 min. Inactivation of 99 percent of the microorganisms in a closed room will require 1-1.5 X 10^6 erg/cm^2. Low temperatures reduce the germicidal properties of UV. Exposure to visible light prior to UV increases resistance to UV.

An unprotected resistant spore would be killed enroute from Earth to Mars, but a spore coated with dead cells or within a speck of dust will be somewhat protected because of the low penetration of UV. There are indications that UV is more effective in destroying organisms on smooth surfaces and in clean air faster than on rough surfaces and in dusty air. Preliminary experiments suggest that dust particles carrying spores in them can partially protect the spores from UV.

149. THE INFLUENCE OF EXPOSURE OF SILVER TO U-V RADIATION ON ITS OLIGODYNAMIC ACTION ON BACTERIA

Bruni, A.

Annali d'igiene, v. 48, pp. 733-742, 1938

(Abstracted in Chemical Abstracts, v. 34, p. 7965[7])

The oligodynamic action of silver on bacteria was activated by exposure of the metal to ultraviolet.

150. INACTIVATION OF MOLDS BY GERMICIDAL ULTRAVIOLET ENERGY

Luckiesh, M., Taylor, A. H., Knowles, T., Leppelmeier, E. T.

Journal of the Franklin Institute, v. 248, no. 4, pp. 311-325, 1949

Doses of germicidal ultraviolet energy required to inactivate 50 and 90 percent of various microorganisms suspended in air and on the surface of culture medium were determined. Organisms in nebulized saliva are easily inactivated, but organisms in dust are several hundred times more resistant. Escherichia coli and saliva organisms display increased resistance to UV with increasing humidity. Mixed airborne organisms sampled in a poultry house appeared less resistant at high humidities. Resistance of airborne mold spores appears to be unaffected by wide variations in atmospheric humidity. Resistance to UV increases in the order bacteria, yeast cells, and mold spores. Aspergillus niger spores are particularly resistant, requiring 1800 and 9000 microwatts/sq cm-min for 50 and 90 percent inactivation in air, and 1300 and 3000 microwatts/sq cm-min for the same degrees of inactivation when irradiated on the surface of a culture medium. The times required to reduce the numbers of airborne Penicillium chrysogenum spores by several germicidal lamp units at

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several positions in a room (ca 17 X 15 X 11 ft) were determined at several relative humidity and air circulation conditions. Spores were inactivated at the highest rates when a fan was used to increase air circulation. Unlouvered lamps irradiating the upper air in the room produced slightly better results than bare 30 w lamps in the center of the room. While 2 hr or more were required for disappearance (primarily by settling out) of 90 percent of the spores without UV, the time was reduced to 10 - 26 min with germicidal lamps. Electrostatic charge held spores to the linoleum floor of the room. Attempts to make such spores airborne were unsuccessful.

151. A COMPARISON OF ULTRAVIOLET AND X-RAY EFFECTS ON *NOCARDIA CORALLINA*

Webb, R. B., Clark, J. B.

Bacteriological Proceedings, p. 49, 1956

The production of unpaired defects by X-ray irradiation of the diploid coccoids of *Nocardia corallina* provides an additional tool for the comparison of the effects of X-ray and ultraviolet irradiation. Successive X-ray irradiation of *N. corallina* coccoids resulted in a significant increase in sensitivity to X-radiation. The ultraviolet dose-survivor response of a culture after a growth cycle following each successive X-ray irradiation showed a close parallel with the corresponding X-ray survival curve. Successive ultraviolet irradiation of *N. corallina* coccoids also produced a significant increase in sensitivity. The X-ray dose-survivor response after a growth cycle following each successive ultraviolet irradiation was similar to that from successively X-ray irradiated cultures. The X-ray survival curves in each case became exponential after the second successive irradiation, whereas the ultraviolet survival curves remained sigmoidal. This difference is suggestive of a combined direct and indirect effect of ultraviolet light. The results are consistent with the theory that the primary end effect of both X-ray

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and ultraviolet irradiation is a change of hereditary material. In each experiment the increase in radiation sensitivity was due to the induction of inheritable unpaired defects. This explanation is not dependent on the mechanism of action of the two radiations.

152. THE MODIFICATION OF ULTRAVIOLET SENSITIVITY OF NEUROSPORA AND BACTERIOPHAGE

Woodward, V. W., Swarup, V., Mora, E., Eisenstark, A.

Bacteriological Proceedings, pp. 49-50, 1956

With the use of agents that modify the effects of radiations, some insight into the mechanism of radiobiological reactions has been gained. Water has been used extensively as such an agent, and it has been demonstrated that organisms of different water content respond differently to irradiations depending upon the source of energy and upon the organism. Neurospora conidia, uv-irradiated dry, yield about five times as many colonial mutants as conidia uv-irradiated in a water suspension. Conidia equilibrated at different relative humidities for 48 hr show a marked increase in sensitivity at or below 15 percent relative humidity (RH). The sensitivity was measured as a function of conidial survival and reverse mutation rate. No detectable differences in sensitivity were observed among the conidial populations kept at 100,50, and 35 percent RH, nor among those kept at 15,5, and 0 percent RH; but a two fold difference between the two groups was observed. The lower RH group was the most sensitive. Phage of Xanthomonas pruni and Escherichia coli showed no such relation between water content and uv-sensitivity. Phage XP4,

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of X. pruni, exhibited an abnormally high degree of resistance to uv at 35 percent RH as compared to other RH levels. With E. coli phage T3, uv-sensitivity was directly proportional to the degree of hydration, an observation at variance with the Neurospora experiments.

153. ULTRAVIOLET MITIGATION OF X-RAY LETHALITY IN DIVIDING YEAST CELLS

Elkind, M. M., Sutton, H.

Science, v. 128, pp. 1082-1083, 1958

X-ray, ultraviolet and visible light were applied in different combinations to dividing nonrespiring haploid Saccharomyces cerevisiae strain SC-7 cells in a study of the nature of the sites sensitive to lethal irradiation. X-ray (55 kv peak) were applied at 30.2 krad/min to achieve doses of 0 - 285 krad. Cells were exposed to ultraviolet (flux, 21 erg/mm^2 sec) for 0 - 140 sec. Small ultraviolet exposures decreased the lethal action of X-rays on the dividing yeast cells whether the UV is applied before or after X-ray exposure. There is quantitative agreement between the protection and reactivation afforded by pre- and post- X-ray exposures to UV. Visible light reverses the UV protection of X-ray lethality as well as UV lethality. The authors support the view that the sites of action of lethal irradiation are chromosomal deoxyribonucleic acid.

154. THE FREQUENCY OF RESPIRATION-DEFICIENT MUTANTS AMONG ULTRAVIOLET-IRRADIATED CELLS OF SACCHAROMYCES PLATED ON GLUCOSE AND ON LACTATE NUTRIENT AGAR

Pittman, D., Roshanmanesh, A.

Bacteriological Proceedings, p. 27, 1959

When respiration-sufficient (AER) yeast cells are exposed to ultraviolet radiation (1 percent survival) and plated on glucose nutrient agar, approximately 40 percent of the survivors form respiration-deficient (aer) variants. The survival of cells from the same irradiated suspension plated on lactate is equal to or greater than the survival on glucose, despite the fact that the aer variants arising on glucose fail to grow on lactate. It has been hypothesized from this observation that the ultraviolet damage producing respiratory deficiency is reversed by the "forced" oxidative metabolism of irradiated cells plated on lactate. A method is described which permits a direct test of this hypothesis: respiration-deficient variants plated on lactate agar remain viable and can be recovered (with 90 percent efficiency) by placing a dry filter paper containing glucose on the surface of the lactate agar. By this technique the number of aer variants among irradiated cells plated on lactate was found to be statistically equivalent to the number of aer variants

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among irradiated cells plated on glucose. No difference in the survival of irradiated aer cells plated on glucose and on lactate was noted using the filter paper technique. Hence the forced oxidative metabolism imposed on irradiated cells plated on lactate does not detectably reverse the aer mutational damage but enhances rather the survival of the parental AER cells over that observed on glucose. The nature of the ultraviolet damage producing respiratory deficiency is discussed in the light of this finding and photoreactivation and genetic studies.

155. MACROMOLECULAR SYNTHESIS IN BACTERIAL RECOVERY FROM ULTRAVIOLET LIGHT

Doudney, C. O.

Bacteriological Proceedings, p. 54, 1959

Numerous investigators have observed that UV induces a lag in DNA synthesis in Escherichia coli. The length of the lag is related to dose. An investigation of the metabolic basis of this lag has been carried out utilizing various inhibitors of RNA, DNA or protein synthesis and also auxotropic strains requiring uracil, thymine or certain amino acids. These studies demonstrate that prior RNA and protein synthesis are required for recovery of the radiation damaged DNA synthetic system. Blocking RNA or protein synthesis during the radiation induced lag period prevents resumption of DNA synthesis. Synthesis does not resume until the RNA or protein block is reversed and the culture goes through a subsequent lag in DNA synthesis during which RNA and protein synthesis take place. The addition of various agents which block RNA or protein synthesis to E. coli strain B cultures after 30 min incubation following UV exposure promote a marked increase in survival (recovery). However, these agents added immediately following exposure do not allow recovery. Evidence will be presented that RNA and protein synthesis

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during the initial period of incubation following exposure is requisite to bacterial recovery but is detrimental to recovery subsequently. Recovery apparently requires (1) synthesis of RNA and protein in reparation of the DNA synthetic system and (2) the prevention of inactivation through unbalanced cytoplasmic growth during the subsequent period required for resumption of DNA synthesis.

156. PHOTOREACTIVATION OF ULTRAVIOLET IRRADIATED ENTAMOEBA HISTOLYTICA

Nakamura, M., Peacock, M. G.

Bacteriological Proceedings, pp. 54-55, 1959

Although numerous studies on the photoreactivation of ultraviolet irradiated bacteria have been conducted, little information is available regarding this phenomenon in protozoa. Furthermore, no such studies have been done with Entamoeba histolytica. Cultures of this protozoan parasite grown with a mixed bacterial flora in an egg slant-liver extract overlay medium were pooled, washed three times in Ringer solution, and made up to a standard suspension containing approximately 50,000 amoebae/ml. Aliquots of this suspension were irradiated with ultraviolet light for varying periods of time at a distance of 15 cm. After the period of irradiation, the amoebae were transferred to egg slant-liver media and incubated at 37 C for 48-72 hr. Amoebic counts were performed in a hemacytometer after the incubation period. Irradiation for 15 to 17 min inactivated the amoebae, i.e., failed to grow and multiply upon subculture. The inactivated amoebae were treated with visible light for 15, 30, 45, 60, and 90 min. Maximum photoreactivation was obtained with a 45 min treatment with visible light. Amoebae irra-

156. Cont'd

diated with ultraviolet light for 18 min could not be photo-reactivated. This is the first recorded evidence of photo-reactivation of ultraviolet irradiated E. histolytica.

157. PHOTOPROTECTION FROM ULTRAVIOLET KILLING IN ESCHERICHIA COLI B

Jaggar, J.

Radiation Research, v. 13, pp. 521-539, 1960

Escherichia coli B cells suspended in phosphate buffer were exposed to light of 3100 to 6000 A prior to exposure to 2537 A ultraviolet light. Photoprotection is a particularly striking effect when the cells are plated on nutrient agar containing sodium chloride. The dose of photoprotecting light that gives optimal photoprotection also kills about 20 percent of the control cells. Photoprotection is important only at low doses of UV while photoreactivation can presumably act on all levels of UV damage. No photoprotection was found from killing by 250-kvp X-rays.

28 references

158. ULTRAVIOLET IRRADIATION OF HOSPITAL STAPHYLOCOCCI

Webb, A. H., Clark, R., Bailey, H. D.

Bacteriological Proceedings, p. 143, 1960

Ultraviolet light has been used in hospitals as a practical means of disinfecting air without taking into consideration its total effects, including mutagenicity, on microorganisms. The possibility exists that such forms as staphylococci which are seeded into the atmosphere and are there subjected to ultraviolet irradiation, receive not lethal but mutagenic doses of ultraviolet. These altered forms then might have lost or acquired characteristics which might have made them more amenable or refractory to control. Strains of Staphylococcus aureus originally isolated from pathologic lesions were categorized as to ability to produce hemolysis, coagulase, penicillinase, production of pigment, ability to ferment mannitol and resistance to penicillin. These strains were then subjected to ultraviolet irradiation for varying periods of time and patterns of survival determined. Subcultures of survivors were plated on trypticase soy agar and random colony isolates tested for conformity with the previously selected markers. Production of pigment, fermentation of mannitol, and resistance to penicillin are apparently controlled by factors resistant to ultraviolet. Hemolysin,

158. Cont'd

coagulase, and penicillinase formation are susceptible to ultraviolet, and organisms which have lost ability to produce these factors are considered to be mutants. These preliminary experiments indicate that large proportions of staphylococci will survive ultraviolet irradiation. Further, if the various markers studied are considered to be phenotypic expressions and are genetically controlled, the genes which control some factors are relatively stable to ultraviolet, others are instable. Resistance to penicillin could not be correlated with production of penicillinase, as strains which had lost ability to produce this enzyme were still resistant to penicillin.

159. ABSENCE OF AN OXYGEN EFFECT ON MUTATION AND KILLING INDUCED BY 2537 A ULTRAVIOLET LIGHT IN OPHIOSTOMA.

Zetterberg, G.

Hereditas, v. 48, pp. 409-416, 1962

(Abstracted in Chemical Abstracts, v. 58, p. 11723c)

Oxygen concentration did not influence mutagenic or killing action of ultraviolet light for exposures up to 18 minutes duration.

160. MUTATIONAL SYNERGISM OF ULTRAVIOLET LIGHT AND CAFFEINE IN ESCHERICHIA COLI

Shankel, D. M., Coupe, B.

Bacteriological Proceedings, p. 55, 1962

When cells of E. coli are exposed to nonlethal amounts of ultraviolet light a significant number of streptomycin resistant mutants are produced. If the mutants are allowed to develop in the presence of caffeine (500 gamma/ml) a ten-fold increase in mutant numbers results. Data will be presented concerning various aspects of this "mutation synergism." The effects of the caffeine are practically completed during the period of mutation stabilization and fixation. If enzymes are extracted from E. coli and added in conjunction with the caffeine, no effect on the phenomenon is observed. Also the addition of normal purine and pyrimidine bases or their ribosides in varying concentrations, or the addition of the metabolic analogues azauracil, azathymine or 5-methyl tryptophan fails to alter the basic effect of the combined UV and caffeine treatment. Short post-irradiation treatments with either puromycin or chloramphenicol, however, completely inhibit the development of the mutations. The development of the mutations is favored by alkaline conditions. Temperature plays an important role in this effect. If the mutations are

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allowed to develop at 37 C or 42 C the regular ten-fold increase occurs. If, however, the mutants express at 30 C the caffeine exerts an inhibitory effect on the number of mutants which will develop.

161. TRANSFER OF ULTRAVIOLET (2537 A) RESISTANCE IN MATING STRAINS OF ESCHERICHIA COLI

Copeland, J. C., Adler, H. I.

Bacteriological Proceedings, p. 59, 1962

It is shown that a UV-resistant Vhf (very high frequency) strain of Escherichia coli may pass the genetic determinants of resistance to a sensitive recipient during conjugation. The progeny produced from such a cross form three classes with regard to radiation sensitivity; one class is like the sensitive recipient; the second class is like the resistant donor; the third class is intermediate between the donor and the recipient. The intermediate class may be further subdivided. The results are consistant with the hypothesis that more than one gene is responsible for the difference in radiation sensitivity between the parental strains. Evidence is presented to show that some of the genes controlling radiation sensitivity are located on the linkage map between the genes controlling proline and histidine synthesis. These results will be discussed in relation to other strains of E. coli.

162. THE INFLUENCE OF IRON ON PIGMENTATION AND RESISTANCE TO ULTRAVIOLET IRRADIATION IN MICROCOCCUS VIOLAGABRIELLAE

Payne, J. I., Campbell, J. N.

Bacteriological Proceedings, p. 59, 1962

Radioresistance has been linked in some bacteria with the possession of pigments. We have found that the non-pigmented bacterium M. violagabriellae can be induced to form a violet-red pigment in the presence of excess iron, thus presenting a unique system for the study of the radioprotection conferred by a pigment. Mutation and selection are not involved. The effect on survival of the presence of pigment, of added iron, and of aerobic or anaerobic incubation were studied after cells were given a constant dose of ultraviolet irradiation. Thirty percent of non-pigmented cells survived the irradiation compared with 70 percent of the pigmented cells. The presence of high levels of added iron subsequent to irradiation allowed the survival of 65 percent of non-pigmented and 75 percent of pigmented cells. Irradiation of the media prior to plating had no effect on viability. Cells grown anaerobically before irradiation exhibited a greater resistance than aerobically grown cells. However, anaerobiosis after irradiation had no effect on numbers of survivors. Organic iron, e.g. hemoglobin, was also protective. The

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results indicate that a site of UV damage in this organism is an iron-dependent system, possibly associated with aerobic respiration. Iron can not only protect against UV damage but may also permit recovery from such damage.

163. CONCERNING THE SITE OF ULTRAVIOLET SENSITIVITY IN MICROCOCCUS VIOLAGABRIELLAE

Payne, J. I., Campbell, J. N.

Bacteriological Proceedings, p. 50, 1963

Studies with ultraviolet filters have established that it is irradiation below wavelengths of 300 mμ which is lethal to M. violagabriellae, probably through damage to the cytochrome system. The site of UV damage was also investigated using various inhibitors. Glutathione and o-cresol had no effect on the susceptibility or recovery of non-pigmented cells to UV damage. When present in the pigment-producing medium, however, they both inhibited pigmentation of the cells and increased the UV sensitivity up to 100 percent over the uninhibited controls, indicating that the site of damage is related at least to the pigment synthesizing mechanism. Although ineffective as an inhibitor of pigmentation, ascorbic acid did increase the UV resistance of non-pigmented cells, possibly by protecting some oxidative-reductive system. Cells grown anaerobically were more UV resistant than aerobically grown cells. However, if nitrate, i.e. an alternative H-acceptor, were incorporated into the medium, UV sensitivity of anaerobically grown cells was restored. Fluoride, a selective inhibitor of glycolysis, had no inhibitory effect

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on aerobic growth but did inhibit 75 percent of anaerobically grown cells; nitrate completely reversed this inhibition. In the presence of a suitable H-acceptor, oxygen or nitrate, the organism is quantitatively more dependent upon a pathway other than glycolysis. Presumably it is a cytochrome-linked electron transfer system with oxygen or nitrate as the terminal acceptor and it is this pathway which is UV sensitive.

164. A NOVEL, OXYGEN-DEPENDENT PHOTOREACTIVATION OF ULTRAVIOLET-IRRADIATED BACILLUS SUBTILIS

Kelner, A.

Bacteriological Proceedings, p. 39, 1964

In the transformable B. subtilis 168, those cells actually able to undergo DNA-mediated genetic transformation are not photoreactivable. Total viable cells of a competent preparation (assay on brain-heart (BH) agar) are photoreactivable, as are cells of a noncompetent suspension grown in BH broth (ms. in press). Photoreactivation (PR) of B. subtilis 168 was compared with three typically photoreactivable organisms. All were tested under identical conditions optimal for B. subtilis. In Streptomyces griseus conidia, BH broth-grown Escherichia coli B/r, and B. cereus, PR was equally high under aerobic or anaerobic conditions (culture bubbled during PR with air or N_2). The relative increase in survival due to PR, in air compared to N_2, was 1.1 for S. griseus, 1.2 for E. coli, and 1.4 for B. cereus. But for BH broth-grown (non-competent) B. subtilis, it was 8.8, showing a requirement for O_2 during PR. With N_2, PR of B. subtilis was almost nil, a 2.4-fold increase in survival. Total viable cells of a competent preparation also required O_2 for PR, but the transformable fraction photoreactivated neither in air nor N_2.

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These experiments strengthened the proposed correlation between transformability and nonphotoreactivability, and further showed that even the PR found in noncompetent B. subtilis was an unusual type.

165. A GENETIC LOCUS AFFECTING CELL DIVISION AND RADIATION SENSITIVITY IN ESCHERICHIA COLI K-12

Adler, H. I., Hardigree, A. A.

Bacteriological Proceedings, p. 39, 1964

Mating experiments between a radiation-resistant high frequency donor strain of E. coli K-12 (HfrH) and a radiation-sensitive recipient (AB1899) have established that a gene (lon) closely linked to the T6 marker influences response to both ultraviolet (2537 A) and ionizing radiation. Mutation at the lon locus results in enhanced radiation sensitivity, the production of a heavy mucoid capsule, and a partial failure of the mechanism that forms the cross-wall. Radiation induces the formation of long, nonseptate filaments in cells containing this mutation. The filaments grow for several hours after irradiation and synthesize nucleic acids at a near-normal rate. Nuclear division, observed by phase microscopy, also proceeds normally. The filaments do not form macroscopically visible colonies on nutrient agar. The frequency of filaments accounts quantitatively for the radiation inactivation of AB1899. Both ends of a filament gain the ability to produce cross-walls and form macrocolonies on nutrient agar which contains pantoyl lactone or omega methyl pantoyl lactone. In this way the lactones completely

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remove the radiation sensitivity induced by mutation at the lon locus. The lactones also interfere with the production of capsular material. We are currently investigating the possible relations between lactone stimulation of cross-wall formation and inhibition of capsule formation.

166. GENETIC ANALYSIS IN ESCHERICHIA COLI K-12 OF A DOUBLE MUTANT SENSITIVE TO RADIATION

Duggan, D. E., Adler, H. I.

Bacteriological Proceedings, p. 39, 1964

Several different gene loci in the linkage group of E. coli K-12 have been shown to be involved in determining the resistance of this organism to radiations. Mutants more sensitive to UV radiation (2537 A) than the parent have been isolated from E. coli K-12 AB1866, itself a UV-sensitive mutant which has lost the mechanism responsible for "dark repair" of UV-induced thymine dimer lesions (Howard-Flanders et al., Proc. Natl. Acad. Sci. US. 48:2109, 1962). One of the mutants isolated was also more sensitive to X-rays than the parent. These mutants, like the parent, form long filamentous cells after exposure to UV. The mutant can be distinguished from the filament-forming "lon" mutant (Howard-Flanders, 1964, Genetics, in press; Adler and Hardigree, 1964, J. Bacterial., in press) since it does not form mucoid colonies when grown on glucose-containing agar. Further, this mutant, unlike the "lon" mutant, exhibits increased resistance to X-rays when grown to stationary phase in a glucose-containing broth. Current experiments are designed to locate the newly mutated gene in the E. coli K-12 linkage group,

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with relation to previously described loci affecting response to radiation and to determine the nature of the action responsible for the increased sensitivity to radiation.

167. STABILITY OF DISSEMINATED AEROSOLS OF PASTEURELLA TULARENSIS SUBJECTED TO SIMULATED SOLAR RADIATIONS AT VARIOUS HUMIDITIES

Beebe, J. M.

Journal of Bacteriology, v. 78, no. 1, pp. 18-24, 1957

Cells irradiated with bactericidal ultraviolet light are protected by water vapor in the atmosphere. The solar UV range is 3000-4000 Å. At a light intensity of 30 μw/sq cm (3400 Å peak response) and 25 percent humidity, the decay rate reached 57 percent per min. As the humidity was raised, the microbial decay rate in an aerosol dropped to 8 percent per min at 95 percent humidity. A linear relationship is shown between percent relative humidity and the amount of protection afforded the Pasteurella cells against bactericidal UV light.

168. SOME BIOLOGICAL AND PHYSICAL FACTORS IN DRY HEAT STERILIZATION: A GENERAL REVIEW

Bruch, C. W.

In "Life Sciences and Space Research", v. II, A Session of the Fourth International Space Science Symposium, Warsaw, June 3-12, 1963, pp. 357-371, North-Holland Publishing Company, Amsterdam, 1964

A review of the significant literature and the status of our knowledge of the biological and physical factors in dry heat sterilization is provided with the author's views and comments. In discussing the effect of exposure environment on dry heat resistance, the enhancing effect of low and ultrahigh vacuum on destruction by dry heat is noted. A vacuum of 50-200 μ Hg at 248 F (120 C) reduced the time required to sterilize spores of Bacillus subtilis var. niger 80 percent and spores of B. coagulans 40 percent compared to sterilization at atmospheric pressure. Spores of these two species were destroyed faster in dry helium gas than in air at 248 F, but the rate of destruction in helium was not as rapid as that obtained with dry heat in a vacuum. Another investigation employing different equipment and B. subtilis strain 5230 obtained greater destruction in air, oxygen and carbon dioxide than in helium or nitrogen,

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Spores of B. polymyxa and B. stearothermophilus strain 1518 have been found to be more resistant to destruction by hot gases of low water content than to superheated steam. The most difficult situation for dry heat sterilization is within solid materials. The presence of many plastics in space probes will make sterilization of these probes difficult.

The enhancing effect of vacuum is restricted to exposed surfaces. Sterilization doses of radiation damage many space probe components. Sub-sterilizing doses of gamma radiation increase the death rate by dry heat of B. subtilis var. niger and B. coagulans in an additive manner readily demonstrated with D values. Dry heat and radiation in combination may prove feasible for sterilizing components which are degraded by sterilizing doses of each agent. Further work in quantitating the increased thermal death rate after irradiation may justify employment of low radiation doses in combination with dry heat for sterilization of space probes or components.

21 references

169. PROBLEMS IN STERILIZATION OF UNMANNED SPACE VEHICLES

Jaffe, L. D.

In "Life Sciences and Space Research", v. II, A Session of the Fourth International Space Sciences Symposium, Warsaw, June 3-12, 1963, pp. 406-432, North-Holland Publishing Company, Amsterdam, 1964

Many spacecraft materials are damaged by common sterilization methods. Exponential kill curves are customary for exposure of microbes to heat, chemical agents and radiation, but deviations attributed to the presence of microbes with differing resistance to the sterilizing agent sometimes lead to a decrease in the rate of kill. Dry heat may be used for spacecraft sterilization, but many spacecraft components are damaged by 24 hr at 135 C. Ultraviolet radiation reaches only directly exposed surfaces while ionizing radiation is more penetrating. Exposure to 5×10^6 rad has been reported to reduce a resistant microbial population to $10^{-5.3}$ of that originally present. A dose of 1.2×10^7 rad should reduce the original count by a factor of 10^{-13}, but this dose will damage many plastics, propellants, glasses and pigments.

Ethylene oxide gas is compatible with the majority of spacecraft components, but penetration is limited and is a function of the material. In order to achieve a probability of 10^{-4} that a single viable organism remains in a surface population of 10^8 organisms, it is necessary to reduce the count by a factor of 10^{-12}.

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This would require about 17 hr assuming killing is exponential. There is evidence that ethylene oxide kill curves are not exponential but level out at 8-18 hr, so that longer exposure produces no further drop in population. There is not sufficient evidence to show that ethylene oxide can produce high probabilities of sterility under spacecraft conditions.

Liquid sterilants damage certain critical spacecraft components. They degrade the electrical properties of electrical conductors. The effectiveness of liquid sterilants of interest seems rather erratic and is sensitive to minor variations in sterilant concentration, quantity, evaporation, hydrolysis, polymerization and storage time as well as substrate nature and cleanliness. The contact times required extend to hours, and the liquid will sterilize only surfaces it touches.

A sporicidal chemical may be added to spacecraft materials during manufacture. Fluids may be sterilized by heat, radiation, and chemicals. Liquids and gases may be passed through bacteriological filters. Because of the likelihood of filter imperfections and filter failure, the assurance of filtration sterilization is only about 10^{-2}.

Air fallout even under clean room conditions contributes 10^{1}-10^{3} organisms/ft^{2} hr detected on culture media. A hood assembly technique in which the hood is sterilized, a positive pressure of filtered air is maintained, and ultraviolet lamps

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are in operation may still provide a 10^{-2} per part chance of contamination from the bags, hands, gloves and air. A liquid sterilant applied to mating surfaces during assembly in the hood should reduce the chance of contamination to 10^{-3}-10^{-4} per part. A glove box previously sterilized with a gas such as ethylene oxide may be best for sterile assembly. Careful control of sterilized subassemblies by a central sterility group is essential to prevent recontamination. A sterilized spacecraft might become contaminated during the launch. Leakage into a closed shroud, and a separation malfunction might introduce contamination.

The radiations in the space environment would not sterilize a spacecraft in flight since they will not reach many areas of the craft because of penetration and dose limitations. Heat sterilization introduced as a deliberate part of the flight sequence might be useful, but it would be difficult to heat a spacecraft evenly in space and avoid interference with instrument calibration. Ablation in a planetary atmosphere or impact on a planetary surface will not sterilize since the fragments will not heat sufficiently. A discussion is presented of problems associated with monitoring, reliability of spacecraft performance, adjustments and repairs and their influence on sterilization.

61 references

170. MODIFICATION OF THE OXYGEN EFFECT WHEN BACTERIA ARE GIVEN LARGE PULSES OF RADIATION

Dewey, D. L., Boag, J. W.

Nature, v. 183, no. 4673, pp. 1450-1451, 1959

Serratia marcescens shows enhanced sensitivity to radiation-the so-called oxygen effect - almost to the full extent when a suspension saturated with a 1 percent oxygen and 99 percent nitrogen mixture is irradiated at about 1000 rad/min. A pulsed linear accelerator can deliver a dose of 10-20 kr 1.5 MV X-radiation in 2 microsec. With such pulses, these bacteria saturated with the same oxygen-nitrogen mixture show the lower sensitivity corresponding to anaerobic irradiation. The first few kilorads probably remove dissolved oxygen from the interior of the cell by radiation-induced reactions. The bacteria will then be under essentially anaerobic conditions since extracellular oxygen cannot penetrate the cell by diffusion in the 2 microsec. Bacteria equilibrated with 5 percent oxygen in nitrogen show nearly the full oxygen effect.

171. CELLULAR RADIOBIOLOGY

Alper, T.

Annual Review of Nuclear Science, v. 10, pp. 489-530, 1960

This extensive review includes many citations concerning modification of radiation response by treatment before, during, and after irradiation. Gol'dat and Alikhanyan (1959) found that doses of 4000 ergs/sq mm of UV, and 36 kr of 60 kv X-rays given separately to Streptomyces aureofaciens spores, reduced the respective surviving populations to 3.7×10^{-3} and to 0.15. If the effects of successive exposures to the two types of radiation were additive, the expected surviving fraction would be 5.5×10^{-4}, provided killing was exponential. It was found, however, that if the X-ray exposure followed UV treatment, the number of survivors was considerably higher than expected on the basis of the two irradiations acting independently. The extent of the increase depended on the time interval separating the exposures; the maximum effect being observed when the X-ray exposure was given one hr after UV, the surviving fraction then being 3.3×10^{-3}. The restoring action of UV was observed both for lethal effects and mutation induction.

Elkind and Sutton (1959) exposed yeast cells to UV either before or after X-rays. If the X-ray survival curves for UV-treated cells were plotted from the level to which the original

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population had been reduced as though they had received the X-ray dose required to reduce the survivors to this level, UV could in most instances be regarded as having a protective action. Large UV doses were more effective in reducing subsequent damage by X-rays if a period of exposure to visible light intervened. Reactivation by visible light after UV does not necessarily bring about a true reversal of UV effects. UV exposure after X-rays was found to be active on the resistant dividing cells. Doses of 400 ergs/mm^2 were most effective, increasing the fraction of cells surviving by a factor of 3 or 4, and thus equivalent to reducing the X-ray dose effectiveness by about one-fourth.

An interdependence of temperature and oxygen effect was reported by Powers, Webb and Ehret (1959). Dry Bacillus megaterium spores irradiated below -150 C did not display any dependence of sensitivity on temperature or oxygen. Above this temperature, sensitivity increased linearly with temperature, but the coefficient of increase was greater if the spores were irradiated in oxygen.

Powers, Kaleta, and Webb (1959) reported that nitric oxide protected dry bacterial spores whether present during or following irradiation, provided the irradiation was done in anoxic conditions.

The effect of 254 mμ UV on bacteria is not influenced by oxygen, but Alper and Gillies (1960) demonstrated that lethality is reduced if UV irradiated Escherichia coli B cells are inoculated

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into nutrient broth and held anoxic at 37 C. Nutritional conditions which increased the rate of growth produced the most radiation damage. Metabolic inhibitors supplied after irradiation reduced the effects of both ionizing radiation and UV. The reverse is true, however, with E. coli B/r, so that this UV resistant mutant and the parent strain become similar in sensitivity under growth inhibiting conditions.

224 references

172. APPLICATION OF HIGH-FREQUENCY ELECTROSTATIC FIELDS IN AGRICULTURE

Ark, P. A., Parry, W.

Quarterly Review of Biology, v. 15, pp. 172-191, 1940

The authors review most of the pre-1940 literature on biological effects of high frequency oscillating fields. Diphtheria, tetanus and botulinus toxins were attenuated by exposure to oscillating currents in the cold. Mycobacterium tuberculosis treated for 30 sec by high frequency currents showed growth retardation from 14 to 24 days after subculturing. Staphylococcus sp. died more quickly than control cultures. Bacteria and fungi can be killed by high frequency currents, depending upon experimental conditions. Escherichia coli is killed most rapidly at 10 mc/sec, 15 and 7.5 mc/sec being of lesser effectiveness. Erwinia carotovora exposed to 10.4 meters in 0.01 percent sodium chloride and then inoculated to agar slants is retarded in growth after 2 min treatment and killed in 2.5-3 min. Suspension density and the time factor were independent in killing of the bacteria. Bacteria on a dried silk thread, however, were not killed in the same study even after 30 min exposure.

Wavelengths of 50 and 100 meters did not affect cultures of the fungi Fusarium batatis, Collybdia dryophila or Sclerotinia bataticola on constant irradiation for 48 and 65 hr at the respective wavelengths. In another investigation, an effect on

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fungi began at 10.4 meters. Wavelengths of 20 and 30 meters were unsatisfactory even with very long exposures. Fusarium solani exposed to a 9 meter field between plates 2.5 cm apart, was inhibited after 5 min and killed after 10 min treatment. A wavelength of 5.6 meters was most effective for fungi. One min treatment inhibited fungus growth while 4-6 min exposures were lethal. The wood destroying fungus Merulius lacrymans was killed in 60 min at a wavelength of 8 meters, while under 4.5 meters 50 min was a lethal dose when the fungus was within or on the surface of wood.

The fungi Sclerotinia libertiana and Botrytis cinerea were unaffected by 1 sec to 30 min exposures in agar media to a wavelength of 2.2 meters between plates 12 cm in diameter, field strength 2000 v, and 5.5 amp in a secondary tank circuit. They were killed, however, by a wavelength of 5.6 meters in 20-25 sec. The agar began to melt when the temperature in the electrostatic field reached 45 C, but 80 C is required in a water bath. Lethal effects of these frequencies began when the temperature of the medium reached 30-40 C. These temperatures are not lethal under ordinary conditions.

It may be possible to disinfect wheat seeds for fungus diseases without seriously reducing the germination of the seeds. Fungi and bacteria may be destroyed in electrostatic fields by heat induced by passage of the electric forces. Perhaps only certain vital cell sites are heated momentarily to such a degree that

172. Con't

death ensues. The principal theories for the phenomena induced by high frequency currents are: heat produced as a result of energy absorption by the treated material (conduction currents); resonance theory, effect of dipoles, and specific effects.

233 references

173. A SPECIFIC EFFECT OF HIGH-FREQUENCY ELECTRIC CURRENTS ON BIOLOGICAL OBJECTS

Nyrop, J. E.

Nature, v. 157, p. 51, 1946

Effects of electric field with minimal heating were explored at 20 mc. Test materials were cooled, and current was modulated to facilitate heat dissipation. When Escherichia coli in liquid medium were exposed to a field strength of 230 v/cm, 99.5 percent of the cells were killed in 7 sec. At 288 v/cm, this degree of killing was accomplished in 4 sec. There was no marked difference whether the treatment took place between 12 and 40 C or 40 and 60 C. In an improved apparatus, 99.6 percent of the bacteria were killed at 205 v/cm in 5 sec and 99.98 percent in 10 sec. This degree of killing by heat alone would require 600 sec at 60 C. Foot-and-mouth disease virus was completely inactivated at 260 v/cm in 10 sec. (temperature not above 36 C) or at 480 v/cm for 2.4 sec. This virus would require 60 hr at 37 C for heat inactivation in the absence of a field. Virus inactivated in an electric field is not satisfactory as a vaccine in contrast to the antigenic activity of virus inactivated by heat. This indicates that electric field and heat have different modes of action. Tissue cultures were

173. Cont'd

killed at 22 v/cm in 300 sec without raising the temperature above 30 C.

174. THE PASTEURIZATION OF AMERICAN CHEDDAR CHEESE BY RADIO-FREQUENCY HEAT

Kosikowsky, F. V., Herrington, B. L., Dahlberg, A. C.

Journal of Dairy Science, v. 32, pp. 790-795, 1949

Young Cheddar cheese may be pasteurized by radio-frequency heat. A 1.3 lb block of cheese could be heated within 1.5 to 2.7 min to temperatures ranging from 117 to 155 F (47-68 C) by means of an oscillator operating at 150 mc at a possible power output of 750 w. The bacterial count 24 hr after heating ranged from 1,500 to 120,000 per g in contrast to 320 million in an unheated control sample. The types of bacteria growing in the cheese were not determined.

175. THE SURVIVAL OF BACTERIA IN HIGH-FREQUENCY ELECTRIC FIELDS

Jacobs, S. E., Thornley, M. J., Maurice, P.

Proceedings of the Society for Applied Bacteriology,

v. 13, pp. 161-169, 1950

The authors attempted to reproduce experiments in which others had indicated the existence of a specific bactericidal action of high frequency fields other than that due to heat generated in the medium. The large lethal effect was of particular interest because of the low power requirement. Cultures included Bacterium coli, Staphylococcus aureus, Pseudomonas fluorescens, Bacillus subtilis, and Sarcina lutea in broth or distilled water suspension. Radio-frequency fields were generated by a low powered apparatus of frequency variable from 1-70 Mc/sec and a 'Metalix' diathermy unit fixed at 44 Mc/sec but of high and variable output. Metal foil electrodes were outside the glass walls of the culture tubes or petri dishes. The volume of fluid treated never exceeded 6 ml and was always entirely within the electric field. Bacterial survival was determined by dilution plate counts made immediately after the field was switched off. Final temperatures were measured at this time.

With the low-powered apparatus there was no significant mortality for B. coli at 1.2, 10, 25, 40, 55, and 66 Mc/sec upon exposure for 6 min between flat electrodes 2 cm apart. Lethality was not observed in another experiment in which B. coli and

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S. aureus were exposed for 5-15 min in a concentric foil electrode system at a higher voltage gradient with electrodes 1.5 and 3 mm apart at 60, 63, and 64 Mc/sec nor at 44 Mc/sec at low and high power levels maintained for 6 and 3 min respectively. Up to 40 percent reduction in count occurred in some experiments conducted with the high-powered generator and 5 mm between the electrodes. The results were not consistent, however, and temperatures reached as high as 43 C in precooled suspensions. Other experiments with the five species at 44 Mc/sec for 30 sec did not achieve complete sterilization. Limitations imposed by the frequencies, power, external electrode arrangement and the failure to induce high mortalities makes it unlikely that the technique will have any practical application in sterilization other than for obtaining efficient uniform heating.

176. THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE MICROBICIDAL EFFECT OF IONISING RADIATIONS

Bridges, B. A., Horne, T.

Journal of Applied Bacteriology, v. 22, no. 1, pp. 99-115, 1959

A summary is given of the physical and chemical factors which modify the microbicidal action of ionizing radiations. The ultimate practical use in radiation sterilization is noted as is the importance of pure research directed at understanding the effects of radiation on living matter. The action of a given factor before or during irradiation may be different from the action of the same factor after irradiation. Most organisms are considerably more resistant to radiation in the absence of oxygen but bacterial spores are much less affected by oxygen. In a commercial radiation sterilization process, the initial number of microorganisms would be kept as low as possible, but resistant organisms may cause a 'tail' on the survival curve. This has occurred with viruses under certain conditions.

Resistance to radiation is generally highest at neutral pH. The pH of the postirradiation medium is also a factor in recovery. Irradiated Escherichia coli B survived better on acid than on alkaline media. Bacteria are most sensitive to X-rays in the logarithmic phase of growth. Vegetative bacteria are more

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resistant when irradiated in the dry state than in wet conditions. There are several reports that bacterial spores are more sensitive when dry than when wet. Vegetative cells are more resistant when frozen, but spores are less affected by freezing. Some spores such as Bacillus subtilis are more resistant in the nonfrozen state, but B. thermoacidurans (coagulans) and Clostridium sporogenes show no difference in resistance whether frozen or not frozen.

Spores and vegetative cells differ with respect to the combined effects of heat and radiation. Bacterial spores are not sensitized by preheating, but irradiation sensitizes them to post-heating. E. coli is sensitized to irradiation by preheating to 45 C, while Saccharomyces cerevisiae is sensitized by preheating at 52.5 C. An irradiation temperature of 52.5 C is more effective than heating the yeast to that temperature before or after irradiation. The radiosensitivity of vacuum dried spores of B. megaterium has been studied from 5 - 404 K. Maximum sensitivity has been reported at 309 K (36 C), minimum sensitivity at 350 K (77 C), and a plateau of temperature independence below about 130 K (-143 C). Partial recovery from radiation damage can occur when certain bacteria are incubated at suboptimal temperature after irradiation.

Oxygen increases the lethal action of radiation on bacteria and is effective at very low concentrations. The relative

176. Cont'd

sensitivities of several bacteria to beta and gamma irradiation is usually of the order: $O_2 >$ air $>$ vacuum $\geqq N_2$. Viruses are exceptions to the generally observed oxygen effect. Coliphage T_1 is protected by oxygen against radiation inactivation. Inert gases at high concentration in the presence of air cause a decrease in radiation sensitivity attributed to displacement of oxygen from cell sites at which oxygen molecules can increase radiation lethality.

Small changes in environmental conditions could lessen the lethal effect of radiation and allow one or two organisms to survive. The conditions at which lethality is greatest must be determined so that sterility may be obtained with the lowest possible and therefore cheapest dose. For food applications, the factors which may best be modified are temperature changes, gas phase composition, and use of chemical additives. These additives have been used only recently with radiation either as sensitizing agents, spore germination inducers, or bacteriostatic agents. No antibiotics have been shown to be radiation sensitizers.

C. botulinum is among the most resistant organisms found in food. For only 1 - 10 such spores/g, the sterilizing dose will be about 3 Mrad. Assuming 10^1 - 10^2 spores/g, 4.8 Mrad will reduce the survivors to 10^{-11} - 10^{-12}/g. In general, 10^5 rad would reduce the microbial population of a food product by a factor of 10^2 or 10^3.

99 references

177. NONCHEMICAL METHODS OF ENZYME INACTIVATION

King, M. E.

Illinois Institute of Technology, Chicago, Illinois

Report No. ARF 3145-4

Final Report No. 4 for April 15, 1959 - April 14, 1960

June 13, 1960, pp. 24

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Heating by electromagnetic energy was evaluated for enzyme inactivation in meat to be subjected to radiation sterilization. Adequate penetration is theoretically obtained with maximum absorption at 200-500 mc. Commercial equipment operates either above or below this range, and units operating at 5-200 mc and 1300 mc were found in initial tests to need extensive modifications to heat large meat samples in a short time. Three 2450 mc electronic ovens were tested for heating ability and effect on proteolysis. Meat was heated and irradiated and the amino acids liberated by proteolytic enzymes after 6 weeks at 98 F were assayed by a modified ninhydrin method. Heating in these ovens followed by irradiation alone was effective, and variations did not offer any advantage. Meat quality was good, and the final temperature

177. Cont'd

did not influence the decrease in proteolysis. Further work is necessary to extend the results to large-scale processing.

178. CONSIDERATIONS ON THE EFFECTS PRODUCED BY SUPERIMPOSED ELECTRIC AND MAGNETIC FIELDS IN BIOLOGICAL SYSTEMS AND ELECTROLYTES

Heinmets, F., Herschman, A.

Physics in Medicine and Biology, v. 5, no. 3, pp. 271-288, 1961

A theoretical analysis is presented for application of combined fields in biological studies. Bacteria moving in a straight line under the influence of an electric field in an electrophoresis apparatus changed direction when a stationary magnetic field was applied perpendicular to the electric field, but continued to move in a straight line following a 20 degree change in direction. In experimenting with combined magnetic and electric fields, it is difficult to obtain uniform fields. Force gradients may be present in an electrolyte, and convection currents appear. Forces present in such fields are too small to produce direct changes in molecules or in structural elements of the cell. The heating effect which results from oscillatory motions of the ions in an alternating electric field is beyond this discussion.

It appears that combined fields at reasonable field strengths would produce a moderate disorganization of cellular growth pattern and not necessarily a direct loss of viability. Abnormal cellular growth may then lead to loss of viability. By application

178. Cont'd

of a high frequency electric field alone, a magnetic induction field is also present, but this is essentially out of phase, and the force component is small. The situation is similar when a high frequency magnetic field is applied. Strong electric fields cause a large energy loss in the system, and a large temperature rise will take place. This can be partially reduced by applying high frequency fields in short pulses. The strongest interaction force on an ionized particle in a liquid occurs when electric and magnetic fields of a travelling wave are in phase and perpendicular to each other, but dielectric heating of the medium or object occurs.

179. EFFECT OF RADIOFREQUENCY ENERGY (2,450 mc) ON BACTERIAL SPORES

Grecz, N., Walker, A. A., Anellis, A.

Bacteriological Proceedings, p. 145, 1964

Spores of Clostridium sporogenes PA 3679 suspended in phosphate buffer (pH 7.0) were exposed to 2,450 mc of radio-frequency energy of about 1 kw power directed into the sample. Temperatures during treatment were maintained at 65, 75, 85, 95, and 100 C by a specially constructed constant temperature microwave device. Microwave heating was consistently more lethal to spores than equivalent conventional heating. The difference between the microwaves and conventional heat was small at 65 and 75 C but became very pronounced at 85, 95, and 100 C. Protein solutions (5 percent beef extract) protected, while acidity (pH 3.1) sensitized the spores. Microwave treatment of spores in the dry state appeared to have no detectable effect on their viability. Treatment with gamma rays at 0.4 Mrad significantly decreased the resistance of spores to subsequent conventional heating and to even a greater degree to microwave heating. Reversing the procedure, i.e., exposing the spores at 85 C for 7 hr to microwaves or conventional heat prior to irradiation had only a small effect on the radiation resistance of spores.

180. SURVIVAL OF ANIMALS IN MAGNETIC FIELDS OF 140,000 Oe

Beischer, D. E.

In "Biological Effects of Magnetic Fields"

Edited by M. F. Barnothy, pp. 201-208,

Plenum Press, New York, 1964

A 2-hr exposure of Neurospora crassa conidia to homogeneous and heterogeneous portions of a 140,000 Oe field produced no mutants. The light emission from Photobacterium fischeri measured by a photomultiplier tube was not changed by a 1-hr exposure to the same magnetic field.

181. INHIBITION OF BACTERIAL GROWTH IN FIELDS OF HIGH PARAMAGNETIC STRENGTH

Gerencser, V. F., Barnothy, M. F., Barnothy, J. M.

In "Biological Effects of Magnetic Fields",
Edited by M. F. Barnothy, pp. 229-239,
Plenum Press, New York, 1964

Nutrient broth cultures of Serratia marcescens and Staphylococcus aureus were grown between the tapered, constant gradient polecaps of a Varian 4-in electromagnet. With S. marcescens the average field strength was 15,000 Oe, with a constant gradient of 2300 Oe/cm throughout the culture medium which was maintained at 27 C. The paramagnetic strength of this field was 34.5 MOe^2/cm or 34.5 par. S. aureus was grown at 37 C. Experiments with this organism using the conditions described previously did not indicate any effect on the growth rate. Polecaps were replaced with others which gave the same average field strength as before, but with an average gradient of 5200 Oe/cm. The gradient in the culture medium ranged from 3000-8000 Oe/cm. The average paramagnetic strength of the field was 78 par, more than twice as strong as that used previously. Bacterial samples were taken at hourly intervals and dilution plated.

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The magnet and control S. marcescens cultures had about the same number of cells and the same growth rate up to 6 hr. At 7 hr the magnet culture showed a lower plate count than the control. This difference was greatest at 8 hr and diminished thereafter up to 10 hr when magnet and control cultures again had about the same number of cells. The growth rate of the magnet culture, therefore, rebounds and surpasses the growth rate of the controls. S. aureus magnet cultures had a higher plate count and higher growth rate than the control from the 3rd to 6th hr at which time growth inhibition began, and reached a maximum at 7 hr, when the magnet culture count fell below that of the control. This difference gradually diminished up to the 9th hr, when magnet and control cultures had approximately the same number of cells. The fact that inhibition was observed in a highly inhomogeneous magnetic field, and inhibition of both organisms occurred in a field of the same average strength but at different paramagnetic strength indicates that inhibition is attributed to a paramagnetic phenomenon in which magnetic dipoles play a role. A mathematical analysis is presented.

182. INHIBITION OF BACTERIAL GROWTH IN HOMOGENEOUS FIELDS

Hedrick, H. G.

In "Biological Effects of Megnetic Fields",

Edited by M. F. Barnothy, pp. 240-245,

Plenum Press, New York, 1964

Bacteria were exposed to a constant homogeneous field generated by a Harvey-Wells model L 128 12-in electromagnet and Alnico V permanent magnets. The cultures included Staphylococcus aureus, Sarcina lutea, and Escherichia coli, considered representative of a biosystem in a space vehicle. Exposed and control nutrient broth cultures were maintained undisturbed at 37 C except when sampled. In electromagnet studies, the paramagnetic strength within the total volume of the exposed cultures within the 4-in homogeneous field was constant at 0.14 MOe^2/cm in a constant homogeneous field of 14,000 Oe. Samples taken hourly during 0-12 and 12-24 hr periods were dilution plated and also stained. A hanging drop slide culture placed between the poles of a permanent magnet was exposed to a constant field of 700 Oe for 36 hr and stained for evidence of morphological changes.

Growth of S. aureus exposed to the 14,000 Oe field was similar to growth of the control for 15 hr. An inhibition of growth noted at the 16th hr continued during the remaining period of exposure. The plate count decreased until the end of the 24 hr

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exposure to about one-fifth that of the control. It was concluded that S. aureus is affected by a constant homogeneous field of 14,000 Oe but is not inhibited if exposure to the same field is interrupted hourly for 3 sec. In the permanent magnet experiments, a disarrangement of cells from the typical grapelike clusters to single isolated cells was observed. The magnetic field may produce a reversal of cell charge creating elemental magnetic fields which repel each other. Neither S. lutea nor E. coli showed significant quantitative differences in cell populations.

183. EFFECTS OF MEASURED ACOUSTIC FIELDS ON MICROCOCCUS PYOGENES VAR. AUREUS AND ESCHERICHIA COLI STRAINS

Dalzell, R. C., Kinsloe, H., Reid, J. J., Ackerman, E.

Bacteriological Proceedings, pp. 52-53, 1956

Buffered suspensions of strains of Micrococcus pyogenes var. aureus and Escherichia coli were exposed to the sound fields of a 10 kc Raytheon magnetostriction oscillator and an 800 kc barium titanate ceramic bowl transducer. Twenty min exposures were made at 15, 25, and 45 C and at various levels of power output. The strain of M. pyogenes var. aureus employed was found to be more resistant to the lethal effects of the sound field than were the strains of E. coli used. Increase in power output resulted in an increase in lethal effect. Temperature effect on rate of killing was not observed in the range 15 to 45 C. Attempts to demonstrate photoreactivation following exposure to a sound field were not successful. The lethal effects of a sound field and ultraviolet radiation were compared employing a radiation susceptible organism, E. coli, strain B, resistant mutant of this strain, B/r, and the resistant strain C 30. Although a marked difference in resistance to ultraviolet radiation was observed, B/r and C 30 proved to be as susceptible to the sound field as did strain B. This suggests that oxidative effects are of

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little significance in the lethal mechanism of vibratory energy. With the 800 kc transducer of r.f. current of 4.5 to 5.0 amperes certain changes were noted in the ability of M. pyogenes var. aureus to produce the pigment typical of the variety.

184 THE BIOLOGICAL EFFECTS OF ULTRASOUND

Wilson, J. W., Curtis, J. C.

U. S. Government Research Reports, v. 39, no. 1, p. 18, January 5, 1964

Contract Nonr. 231400, Task 100 007

AD - 416 751 1963 11 pp.

Final Report

Ultrasonic radiation effects on yeast and mammalian tissues are described.

185. SHOCK, VIBRATION, ACCELERATION AND ULTRASOUND EFFECTS ON BIOLUMINESCENT FUNGI

Berliner, M. D., La Rochelle, M. F.

Bacteriological Proceedings, p. 5, 1963

Cultures of the luminescent fungi, Panus stipticus and Armillaria mellea were exposed to a series of shock, vibration, acceleration tests as anticipated in the sending and re-entry of a space probe, as well as to a range of ultrasonic vibrations. Change in the light emission intensity serves as an indicator of physiological changes in the organisms. P. stipticus cultures were unaffected by all tests. A. mellea responded as follows: 1. Shock - 100 g, 6 millisec 40 percent increase in light emission. 2. Random vibrations - 15-2 KC, 1 min - 1 hr. 30 min and 1 hr exposures yielded light increases up to 250 percent. 3. Acceleration - 2-20 g, 5 min. All exposures yielded light increases of about 150 percent. 4. Ultrasound - 20,000 cycles/sec for 1 sec - 10 min. Stepwise increases in light emission up to 400 percent at 1, 3, 5, 10 min. All increases in light emission were transient and intensities returned to pretest levels in 5 hr. There was no visible cell damage or changes in growth rate. Stimulation is assumed to be due to release of enzymes and substrates into the medium. The 2 species may react differently because of their mode of growth, aerial for P. stipticus and

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submerged for A. mellea. NASA contract NASw-389.

186. COMPLEMENTARY EFFECTS OF THERMAL AND IONIZING ENERGY

Licciardello, J. J.

In "Exploration in Future Food - Processing Techniques",

pp. 37-45, S. A. Goldblith, Editor,

The M. I. T. Press, Cambridge, Massachusetts, 1963

Four to five million rad are required to sterilize foods by means of ionizing radiation with the same probability of spoilage by Clostridium botulinum as is achieved by thermal processing. In an effort to lower the radiation dose to improve the quality of the product, irradiation has been coupled with other lethal treatments such as ultrasonic energy and antibiotics. The use of thermal energy with ionizing radiation seems most promising. The complementary effect occurs only when the irradiation precedes the heat treatment and has also been observed with ultraviolet radiation and also with visible light in photodynamic dye-sensitized cells. It is generally believed that irradiation lowers the thermal requirement for protein denaturation.

The influence of environmental conditions during irradiation on the heat sensitization of C. sporogenes P.A. 3679 spores, Bacillus subtilis spores, and Salmonella typhimurium was investigated. When spores in neutral phosphate buffer were irradiated over the range 2-10 X 10^5 rep in air at room temperature and then heated at 90 C, it was found that B. subtilis was heat - sensitized by

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the irradiation to a lesser degree than C. sporogenes. The extent of heat-sensitization depends upon the substrate present during irradiation. Experiments with C. sporogenes and C. botulinum showed less heat sensitizing effect in complex organic media than in neutral phosphate buffer. In general, the degree of radiation-induced heat sensitization was not significantly changed under air or 1 mm oxygen tension; over the pH range 4.5-7.0; whether irradiation was carried out in the frozen state or 68 F; and was independent of spore concentration. C. sporogenes at about 153 F and 600,000 rad showed a significant increase in heat sensitization.

The lethal effect of simultaneously applying Co-60 gamma radiation and heat to Salmonella typhimurium suspended in liquid whole egg was compared with the lethality which occurred when irradiation was followed by an equivalent amount of heat. When the irradiation dose ranged from 0-60,000 rad and the temperature during or following irradiation was 130 F, a marked difference in survival was observed on comparing the two processes at increasing radiation doses. At 60,000 rad there were a hundred times more survivors when heating followed the irradiation (at 32 F) than when heating and irradiation were simultaneous. Similar experiments at 100 F demonstrated that survival at 50,000 rad was two-fold and at 100,000 rad four-fold greater when heating followed irradiation rather than concurrent with irradiation. Comparison

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of survival data from experiments at 45,000 rad, 130 F and 50,000 rad, 100 F indicates that the 30 F increase in temperature increased lethality 300- and 40- fold respectively for simultaneous irradiation and heating and irradiation followed by heating, if one assumes the irradiation in both instances to be equivalent.

12 references

187. EXPOSURE OF MICROORGANISMS TO SIMULATED EXTRATERRESTRIAL SPACE ECOLOGY

Silverman, G. J., Davis, N. S., Keller, W. H.

In "Life Sciences and Space Research", v. II, A Session of the Fourth International Space Sciences Symposium, Warsaw, June 3-12, 1963, pp. 372-384, North-Holland Publishing Company, Amsterdam, 1964

The viability of spores of five test species and organisms in soil was determined after exposure to the temperature range -190 to +170 C for 4-5 days in ultrahigh vacuum. Spores were also exposed to Co-60 gamma radiation following ultrahigh vacuum treatment. Spore populations in vacuum at -190 to +25 C were quantitatively similar to preparations maintained at room temperature and atmospheric pressure in a desiccator. Survival of Bacillus megaterium, B. stearothermophilus, and Clostridium sporogenes in ultrahigh vacuum at 60 C was significantly lower than survival of B. subtilis var. niger and Aspergillus niger. A. niger survivors were detected in a qualitative assay after exposure to 107 C in vacuum, but none were viable at 120 C. The survival pattern of these spores at 90 C and atmospheric pressure was notably different than in vacuum. Viable B. stearothermophilus, C. sporogenes, and A. niger spores could not be recovered after only one day at 90 C and atmospheric pressure. Under the same conditions, B. subtilis var. niger spores were recovered daily in two experiments conducted

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for 5 and 7 days.

Garden soil was maintained in ultrahigh vacuum for 5 days at various temperatures. Mesophilic aerobes and anaerobes, molds and actinomycetes were recovered in quantitative assays at 90 C; molds were not detected at 110 C. At 170 C, a qualitative assay demonstrated survival of members of all but the mesophilic anaerobe group. A soil from the Mohave desert also had low numbers of living organisms after 4.5 days at 170 C in vacuum.

The gamma radiation resistance of extremely dry spores which had been in ultrahigh vacuum was compared with the radiation resistance of similar spores stored over silica gel. B. stearothermophilus was much less radioresistant after ultradrying than in neutral phosphate buffer saturated with nitrogen. B. stearothermophilus, B. megaterium, and C. sporogenes exposed to vacuum but irradiated in air were appreciably more sensitive to radiation than spores irradiated in vacuum. Spores dried over silica gel and irradiated in air were more resistant than the ultradry spores also exposed to air during irradiation and were of equivalent resistance to ultradry spores irradiated in vacuum.

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